The effects of a non-competitive NMDA receptor antagonist FR115427 on LTP, spontaneous behaviour and performance in the water maze

by

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In accordance with the requirements of the University of Edinburgh regulation, this thesis has been composed by myself and this work presented herein is my own.

Hirohisa Nakada

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Abstract

The effects of *N*-methyl-D-aspartate (NMDA) receptor antagonists on learning behaviour have been studied extensively as they block long-term potentiation (LTP), the suspected neural substrate for memory storage mechanisms. However, NMDA receptor antagonists also induce prominent changes in motor behaviour and so it is ambiguous whether the learning impairment induced by these drugs are due to a block of LTP or a behavioural abnormality.

Competitive NMDA receptor antagonists are not fully satisfactory pharmacological tools in examining the above question because the dose response of both effects are too close to distinguish. On the other hand, the non-competitive NMDA receptor antagonists like MK-801 (dizocilpine) exhibit distinguishing behavioural effects but they cause ataxia that is so severe that they can hardly be used in behavioural experiments.

In an attempt to resolve this issue, the drug chosen in this thesis was a novel non-competitive NMDA receptor antagonist, FR 115427 ((+)-1-methyl- 1-phenyl-1,2,3,4-tetrahydroisoquinoline hydrochloride). Although its binding affinity to the NMDA receptors is about 10 times weaker than that of MK-801, it induces relatively mild ataxia and shows a faster time course of kinetics. This allows testing in a wider range of doses; it also exhibits clear-cut time effects.

Three types of procedure are used in this thesis: open field activity tests, *in vivo* LTP examinations and water maze experiments. The open field activity tests showed that FR115427 induced a characteristic motor syndrome: ataxia, head weaving, body rolling and hyperlocomotion. These effects occurred with a potency of approximately 30 ~ 100 times weaker than those produced by MK-801. With FR115427, indices for

ataxia and head weaving reached a peak within 30 min post injection and began to decline after 40, whereas the peak indices with MK-801 was less well defined and lay between 40 and 60 min.

LTP examinations in anaesthetised rats showed that a 10 mg/kg dose of FR115427 completely blocked LTP which was induced 90 min post injection but not if it was induced 30 min post injection. This suggests a late onset effect on LTP by the drug. MK-801 was too toxic at doses required to show a significant effect in anaesthetised animals.

The water maze experiments showed that FR115427 impaired the spatial learning at a lower dose (3.2 mg/kg) than that was required to block LTP. A comparison of the learning effect between a short interval (20 min) experiment and a long interval (90 min) experiment revealed that the learning effect of the drug had an early onset. MK-801 did not show any clear-cut time dependent effects at the doses tested.

In conclusion, the action of FR115427 on LTP was temporally and dose dependently differentiated from that on spontaneous behaviour and learning. It is suggested that non-competitive NMDA receptor antagonists impair spatial learning by an unknown mechanism that need not be related to their action on LTP.

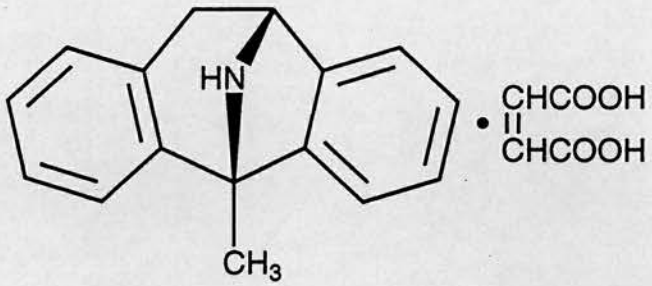
Contents

Chapter 1
Introduction

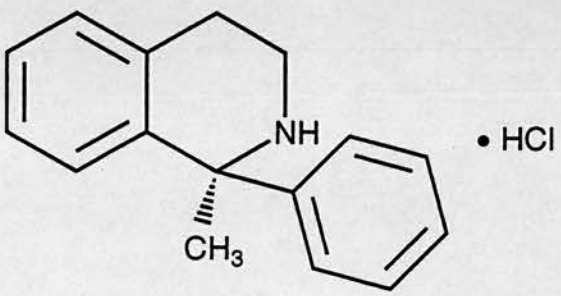
The novel isoquinoline derivative (+)-1-methyl-1-phenyl-1, 2, 3, 4-tetrahydroisoquinoline hydrochloride (FR115427, Fig. 1-1), synthesized by Fujisawa Pharmaceutical Co., Ltd., has been characterized as a specific non-competitive NMDA receptor antagonist (Hodgkiss et al., 1993; Sherriffs et al., 1993). It has been found to prevent neuronal death following ischaemic insult in gerbils (Nakanishi et al., 1994) and in rats (Katsuta et al., 1995). The pharmacological analysis of this compound is expected to give us useful informations about the physiological role of NMDA receptors and the clinical side effects in the therapeutic use of this compound for stroke. From this point of view, this thesis investigated, the effect of FR115427 on synaptic transmission and synaptic plasticity *in vivo*, spontaneous behaviour and a type of learning thought to depend on NMDA receptors.

1.1 NMDA receptor

Excitatory action of glutamate in the brain of rats, dogs and human(s) was originally reported by Hayashi in 1954. Nowadays, glutamate and related excitatory amino acid receptors are accepted as the main transmitter receptors mediating synaptic excitation in the mammalian central nervous system (Watkins and Evans, 1981; Collingridge et al., 1983; Monaghan et al., 1989). Four classes excitatory amino acid receptors are recognized to date, and all of which bind glutamate with



MK-801



FR115427

Fig. 1-1 Chemical structues of (+) MK-801maleate (dizocilpine) and (+) FR115427 HCl

high affinity (Monaghanet al., 1989; Iversen, 1995). Three of them are ionotropic glutamate receptors functioning as ligand-gated cation channels and are traditionally defined by their selective agonists: N-methyl-D-aspartate (NMDA) receptors, kainate receptors, and alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptors. The forth class of receptors is a family of G protein coupled metabotropic glutamate receptors. Recent advances, achieved using molecular cloning and mutagenesis coupled with functional analysis have revealed a high degree of molecular diversity in the subunits of the ionotropic receptors and metabotropic glutamate receptors, (Seeburg, 1993; Hollmann and Heinemann, 1994; Nakanishi and Masu, 1994; Pin and Duvoisin, 1995).

Among a variety of glutamate receptors, considerable attention has been focused on the NMDA receptors because it has been found to be implicated in neuropathological cell death (Meldrum and Garthwaite 1990; Olney 1990) and synaptic plasticity and learning (Morris et al., 1986a).

1.1.1 Competitive antagonists for NMDA receptors

The key step in the analysis of NMDA receptors has been the development of specific antagonists. D(-) isomers of 2-amino 5-phosphonopentanoate (AP5) (Davies and Watkins, 1982; Evans et al., 1982) and 2-amino-7-phosphonoheptanoate (AP7) (Perkins et al., 1982) were developed as specific competitive NMDA antagonists and are useful as the pharmacological tool for investigating and identifying the role of NMDA receptors in the CNS. These compounds are also the bases for development of more potent antagonists. An AP7 analogue with a conformationally restricted structure, 3-((±)2-carboxypiperazin-4-yl) propyl-1-phosphonate (DL-CPP) was developed to show higher affinity for NMDA receptor

than AP5 (D-isomer of CPP was found to have higher affinity than L-isomer later). A cyclic analogue of AP5, cis-4-phosphonomethyl-2-piperidine carboxylate (CGS19755) is about equipotent with DL-CPP. [^{3}H] D-AP5 and [^{3}H] CPP label a single population of sites with K_D values of 0.5 μM and 0.3 μM respectively and B_{max} values of around 4 pmol/mg protein in tests using synaptosomal (P_2) fraction of rat cerebral cortex. K_i values of glutamate and NMDA for inhibition of [^{3}H] D-AP5 binding are 0.9 μM and 11 μM respectively (Olverman and Watkins 1989).

1.1.2 Recognition sites for modulators of NMDA receptors

In addition to the agonist recognition site, the NMDA receptor/channel complex has several regulatory sites. Lodge et al.(1989), Wong and Kemp (1991) enumerate the proposed regulatory sites as follows.

(i) Magnesium site: At the resting potential, Mg^{2+} blocks the NMDA ion channel by binding to a site deep within the ionophore at a concentration well below that normally present in extracellular fluid. This confers a unique voltage dependency on the NMDA receptor-mediated response, which is evoked very little at low frequency synaptic activation, but becomes apparent when the membrane is depolarised (above -50 ~ -30 mV) for more prolonged periods (e.g. during repetitive high-frequency synaptic activation).

(ii) Phencyclidine (PCP) site: The dissociative anaesthetics (e.g. PCP, ketamine) and MK-801 (see below) have high affinity in a stereoselective manner for a single population of sites whose affinity correlates well with their potency as blockers of NMDA-induced depolarization in rat brain. The selective binding of [^{3}H]-MK-801 is markedly enhanced by NMDA receptor agonists and inhibited by

competitive NMDA receptor antagonists. The non-additive nature of combinations of ketamine with both competitive antagonists and Mg^{2+} suggests that ketamine acts at a site distinct from the NMDA recognition site and from the magnesium site. The requirement of repeated application of NMDA to fully develop the block of the NMDA response by MK-801, a phenomenon called use-dependency, suggests this group of compounds antagonize NMDA by entering and blocking the open channel.

(iii) Glycine site: Glycine potentiates the NMDA response by increasing channel-opening probability in a voltage independent manner. Only very low concentrations of glycine are required for this effect (EC_{50}: 100~300 nM) about ten fold lower than those required to activate the inhibitory, strychnine sensitive receptor. Glycine has been suggested to bind to an allosteric site within the NMDA receptor and act as a co-agonist, i.e. agonist binding at both the glutamate recognition site and glycine site is required for channel activation. (R)-α-amino acids, such as D-alanine and D-serine, are found to be potent agonists. Kynurenic acid analogues, such as 5,7-dichlorokynurenic acid or L-689,560, are developed as specific antagonists for this sites (Leeson and Iversen 1994).

(iv) Zinc site: Zn^{2+} produces selective and non-competitive antagonism of NMDA receptors in cultured neurons. Its action is distinct from that of Mg^{2+} in that the reduction of NMDA currents is relatively less voltage-dependent, but is still due to reduced channel open time. Zn^{2+} also reduces binding at the PCP site in a manner different from all other classes of NMDA antagonist. This action is mimicked by high doses of some tricyclic structures such as desipramine and promethazine although, electrophysiologically, the tricyclics show some voltage dependence.

(v) Polyamine site: Polyamines, such as spermidine potentiate MK-801/PCP binding. This may be part of a positive feed back system as NMDA also stimulates the activity of ornitine decarboxylase, the enzyme responsible for the synthesis of such polyamines. The neuroprotective agent ifenprodil may act at this site.

1.1.3 Calcium permeability

The important role of NMDA receptors in central nervous system is mediated by Ca^{2+} entering through its associated channel. The Ca^{2+} permeability of NMDA receptor channels is shown by voltage-clamp studies of cultured neurons injected with a Ca^{2+}-sensitive dye, which directly indicates Ca^{2+} influx after NMDA applications in a manner independent of the voltage-dependent Ca^{2+} channels (MacDermott et al., 1986; Mayer et al., 1987). Furthermore, the reversal potentials of NMDA-induced currents are appropriately altered by changes in the extracellular concentration of calcium (Mayer and Westbrook, 1987; Jahr and Stevens, 1987; Ascher and Nowak, 1988).

The increase in intracellular ions is thought to initiate the biochemical process responsible for NMDA receptor-induced plasticity (long-term potentiation) (it is further discussed in section 1.3) and NMDA receptor mediated excitotoxic cell death (Rothman and Olney, 1987).

1.1.4 NMDA receptor heterogeneity

The development of molecular biological techniques achieved cloning of complementary DNA encoding a vast variety of subunits of AMPA, Kainate, NMDA and metabotropic glutamate receptors of rats (Nakanishi, 1992; Seeburg, 1993). A numbers of mouse glutamate receptor subunits have also been cloned and characterised by Mishina's group (Kutsuwada et al., 1992). It is now known that the ionotropic glutamate receptors form a distinct ligand-gated ion channel family and the size of their subunits is about twice of that of nicotinic acetylcholine receptor subunit. Each subunit of glutamate receptor probably three or five transmembrane

domaines, whereas nicotinic receptor subunit has four (Hollmann et al., 1994; Galen Wo and Oswald, 1995; Bennett and Dingledine 1995).

According to the reviews by Seeburg (1993) and Nakanish and Masu (1995), NMDA receptors of rats can be reconstituted as heteromeric structures from two subunit types, the NMDAR1 (NR1) subunit and one of four NMDAR2 subunit (NR2A, NR2B, NR2C and NR2D). Expression of NR1 alone, or combined expression of NR1 with one of NR2 subtypes forms an active receptor complex in *Xenopus* oocytes. The resulting complexes display characteristic NMDA channel properties, including Ca^{2+} permeability, the requirement of both glycine and glutamate (or both glycine and NMDA) to activate the channel, sensitivity to Mg^{2+} in a voltage-dependent manner and sensitivity to the channel blocker MK-801. The different combinations of the various subtypes show different affinity for various agonists and antagonists, and varying kinetics of responses and stimulatory effects of glycine. *In situ* hybridization analysis revealed that NR1 mRNA is found at a high level in most areas of rat brain while the mRNA of NR2A, B, C and D shows distinct spatial patterns of expression (Monyer et al., 1992; Ishii et al., 1993). These expression patterns coupled with a functional difference in subtypes of subunits could provide the molecular basis for the generation of the heterogeneity in the physiological and pharmacological properties of NMDA receptors proposed to occur in different neuronal cells and brain regions (Monaghan et al., 1988; Monaghan, 1991; Gonzales, 1992). A further variation of NMDA receptors due to alternative splice forms of the NMDAR1 subunits mRNA is also reported by several groups including Hollmann et al. (1993) and Laurie et al. (1995). They reported that splice variants of NMDAR1 show heterogeneity in functional properties and regional distribution in the brain. This multiple alternative splicing may be an alternative basis for the known heterogeneity of endogenous NMDA receptors.

1.2 MK-801 and FR115427 : PCP like NMDA receptor antagonists

1.2.1 MK-801

MK-801 or Dizocilpine ((+)-5-methyl-10, 11-dihydro-5H-dibenzo [a, d] cyclohepten-5, 10-imine maleate) (Fig. 1-1) is a potent NMDA antagonist which has the therapeutic advantage of good CNS penetration across the blood-brain barrier.

This compound was discovered originally by Clineschmidt et al. (1982a, b and c) as a potent anticonvulsant agent with anxiolytic and sympathomimetic properties. Subsequently, Wong et al.(1986, 1988) found that radiolabeled [^{3}H]-MK-801 bound to a specific population of receptor sites in rat brain membranes, and these appeared to be associated with a glutamate receptor of the NMDA type. [^{3}H]-MK-801 binding was displaced by structural analogues of MK-801, phencyclidine(PCP), ketamine, and (+)SKF-10,047. Foster and Wong (1987) reported that high-affinity [^{3}H]-MK-801 binding requires the presence of micromolar concentrations of L-glutamate or other NMDA agonists, suggesting a functional link between the MK-801 binding site and the agonist binding site. Later, [^{3}H]-MK-801 binding was also found to be enhanced by glycine and other compounds that act at the strychnine-insensitive glycine modulatory site on NMDA receptors (Reynolds et al., 1987; Wong et al., 1987).

Electrophysiological studies using slices of rat cerebral cortex showed that MK-801 antagonized the depolarizing actions of NMDA, leaving response to quisqualate or kainate unchanged (Kemp et al. 1986). Moreover, a near perfect correlation was found between the ability of MK-801, PCP, ketamine and SKF10,047 to block NMDA responses and to displace [^{3}H]-MK-801 binding (Wong et al., 1986). The blockade of NMDA responses in rat cortical slices by MK-801

was found to be accelerated by repeated agonist administration illustrating the use-dependent nature of antagonism by MK-801. (Kemp et al., 1986; Woodruff et al., 1987)

Therefore, the antagonist effects of MK-801 are regarded as non-competitive and agonist-dependent.

1.2.2 FR115427

(+)-1-methyl-1-phenyl-1, 2, 3, 4-tetrahydroisoquinoline hydrochloride (FR115427, Fig. 1-1) is a novel MK-801 analogue synthesized in the New Drug Research Laboratories of Fujisawa Pharmaceutical Co., Ltd. Osaka Japan.

The pharmacological profile of FR115427 has been examined *in vitro* by Hodgkiss et al. (1993) and Sherriffs et al. (1993).

Ligand binding studies by Hodgkiss et al. (1993) and Sherriffs et al. (1993) using rat cortical membrane have revealed that:

(a) [^{3}H] MK-801 binding was inhibited by MK-801 and FR115427 with Ki values of 3.14 and 43.3 nM respectively in Hodgkiss's data and, 3.57 nM and 35.4 nM respectively in Sherriffs's data in the presence of L-glutamate (10 μM). Those data show FR115427 has about a 10-fold lower affinity than MK-801.

(b) The Ki values for non-competitive NMDA receptor antagonists (PCP, ketamine, N-allylnormetazocine) in the [^{3}H] FR115427 and [^{3}H] MK-801 binding assay exhibit a close correlation ($r = 0.964$) (Sherriffs et al. 1993).

(c) The affinity of FR115427 for [^{3}H] MK-801 was enhanced by addition of L-glutamate and/or glycine however the extent of enhancement was less than that for MK-801 (Sherriffs et al. 1993).

The electrophysiological study (Hodgkiss et al. 1993) has revealed that:

(d) The depolarization induced by NMDA was inhibited by FR115427(1 μM) in cortical wedge preparations.

(e) The Schild plot showed a slope of 1.21~1.6 (greater than 1) and a pA_2 value of 6.45~6.6.

(f) The electrophysiological responses to the non-NMDA agonist AMPA were unaffected by FR115427.

(g) FR115427 showed use-dependent blockade of NMDA induced depolarization and antagonism could not be overcome by increasing the dose of NMDA.

(h) Intracellular recording in hippocampal pyramidal cells has revealed that FR115427 blocks a second slow component but not the first fast component of excitatory postsynaptic potentials (EPSPs) elicited by synaptic stimulation.

The above binding studies show that FR115427 competitively interacts with MK-801 binding sites and electrophysiological experiments confirm that FR115427 functionally acts as a non-competitive NMDA receptor antagonist.

In vivo study revealed that:

(i) FR115427 completely suppresses NMDA-induced convulsions in mice at doses 0.32 μg/mouse i.c.v., 32 mg/kg i.p. or 100 mg/kg p.o. (ED_{50} values reveal a 10 times weaker potency of FR115427 than MK-801 in all administration routes) but not quisqualate or kainate induced convulsions (Nakanishi et al., 1995).

(ii) Pretreatment with 10 mg/kg FR115427(i.v.) or 1 mg/kg of MK-801(i.v.) protects neuronal damage in the hippocampal CA1 region of gerbils following 5 min bilateral carotid artery occlusion (Nakanishi et al 1994).

(iii) 10 mg/kg FR115427 (i.p.) significantly reduced the brain damage while 1 mg/kg (i.p.) of MK-801 was effective in rat MCA occlusion model (Katsuta et al., 1995)

These evidences prove that FR115427 is active as an NMDA receptor antagonist *in vivo* as well as *in vitro* and its potency is about 10 times less than that of MK-801.

Although the fundamental features of FR115427 as an non-competitive NMDA receptor antagonist are similar to that of MK-801, FR115427 may not be just a weaker analogue of MK-801. For example:

The EC_{50} values for stimulation of [^{3}H] FR115427 binding by L-glutamate and glycine were 2~4 times lower than that for [^{3}H] MK-801 binding (Sherriffs et al., 1993)

FR115427 exhibits higher stereoselectivity than MK-801 in the affinity for NMDA receptors (Sherriffs et al., 1993; Nakanishi et al., 1995).

The ratio of the dose that induces locomotor activity to the anti convulsant dose for FR115427 is higher than the ratio for MK-801(Nakanishi et al., 1995). Thus, FR115427 induces less intense behavioural side effects than MK-801 relatively to their anticonvulsant potencies.

Although it is not clear whether all these differences between FR115427 and MK-801 are related to each other, the less behavioural side effects may be an advantage of FR115427 in utilising it in the study of learning and memory. As is discussed later, the behavioural side effects of MK-801 limits the use of this drug in the study of animal learning models.

1.3 Long-term potentiation

The recent attention to the involvement of NMDA receptors in learning is greatly due to its capacity to their role in inducing hippocampal long-term potentiation (LTP) (Collingridge et al., 1983).

LTP is a relatively long lasting increase in synaptic efficacy at monosynaptic junctions, occurring as the result of an afferent fibre tetanisation. LTP was first described by Bliss and Lømo (1973) who demonstrated that repetitive high frequency stimulation of medial perforant pathways of the hippocampus, in the anaesthetized rabbit, induced a potentiation of synaptic transmission lasting up to 10 hr. Subsequent experiments with chronically implanted electrodes showed that LTP could last for days and weeks at a time (Bliss and Gordner-Medwin, 1973; Douglas and Goddard, 1975).

1.3.1 NMDA receptors and LTP

The role of NMDA receptor in the induction of LTP was first demonstrated in the CA1 region of hippocampal slices of rat. It was found that the competitive NMDA receptor antagonist AP5 (2-amino-5-phosphonopentanoate) reversibly prevented induction of LTP of the population spike in the Schaffer/commissural pathway, without affecting the low frequency response either before or after LTP was induced (Collingridge et al., 1983). Subsequently, AP5 was found to prevent induction of LTP of dendritic EPSPs in CA1 (Collingridge et al., 1987) and has been found to prevent induction of LTP in the rat dentate gyrus *in vivo* whilst having little or no effect on the normal low frequency response (Morris et al., 1986a; Errington

et al., 1987). The non-competitive NMDA antagonist, MK-801, has also been found to block the induction of LTP *in vitro* (Coan et al., 1987) and *in vivo* under certain conditions (Abraham and Mason, 1988; Morimoto et al., 1991).

The reason that tetanic stimulation is required to induce LTP under standard experimental conditions may be solely to keep the cell at a depolarised level to relieve the voltage dependent Mg^{2+} block of NMDA receptors. Treatments that allow NMDA receptor activation, such as a Mg^{2+} -free medium, depolarization of cell membranes and high intensity stimulation, also induce LTP in an AP5-sensitive manner. Even if AMPA receptors are blocked by CNQX (6-cyano-7-nitroquinoxaline-2, 3-dione), LTP can be induced by the tetanus stimulation. Therefore, NMDA receptor activation is critical for the induction of LTP in CA1 (Collingridge and Davies 1989).

For the most part, perforant path and Schaffer collateral LTP appear to be quite similar, whereas mossy fibre to CA3 synapses exhibit a substantially different, NMDA receptor-independent form of LTP (Harris and Cotman, 1986; Zalutsky and Nicoll, 1990; Staubli et al., 1990). In the following part of this thesis, NMDA receptor dependent LTP will be mainly discussed.

1.3.2 Mechanism of LTP

The high permeability of the activated NMDA receptor-gated ion channel to Ca^{2+} ions results in a large increase of intracellular Ca^{2+} levels in dendritic spines which can then trigger long lasting molecular events. The critical role of Ca^{2+} is supported by the observation of the calcium dependency of LTP (Wigström et al. 1979), the blockade of LTP by intracellular injection of calcium-chelating EGTA (Lynch et al. 1983), the increase of calcium deposits in dendrites after induction of

LTP (Kuhnt et al. 1985) and induction of LTP-like synaptic enhancement by increasing intracellular Ca^{2+}.(Turner et al. 1982, Reyman et al., 1986). LTP is also found to be associated with increased phosphoinositide turnover (Lynch et al. 1988) and arachidonic acid metabolites (Williams and Bliss 1988). These system may interact in the activation of Ca^{2+} dependent enzymes such as protein kinase C, Ca^{2+}-calmoduline-dependent kinase II and Ca^{2+} dependent protease that may lead to genomic events such as induction of c-fos, jun-B and zif/268 expression (Madison et al. 1991).

At least some of the critical events responsible for triggering LTP occur in the postsynaptic cell but the locus for the persistent synaptic modulation has been controversial especially since the proposal of a pre-synaptic component of LTP (Malinow and Tsien, 1990; Bekkers and Stevens, 1990). Recently, the analysis of the trial to trial amplitude fluctuation of synaptic signals in CA1 hippocampus has reconciled the different views (Kullman and Nicoll, 1992; Liao et al., 1992). These new analyses show an increase both in the number of quanta released and in quantal amplitude consistent with combined pre- and post-synaptic modification (increase in neurotransmitter release and augmentation of postsynaptic response) after induction of LTP.

If part of the mechanism for the expression of LTP resides within the presynaptic cell, the postsynaptic cell must communicate with the presynaptic terminal, perhaps by releasing some factor. The prominent candidates for such messengers are arachidonic acid (Williams et al. 1989), nitric oxide (Bohme et al., 1991; O'Dell et al., 1991; Schuman and Madison, 1991; Haley et al., 1992) and carbon monoxide (Zhuo et al., 1993; Stevens and Wang, 1993).

Although the detailed mechanisms of LTP are still far from fully understood and are based, in many respects, on still unsettled experimental results, two properties of NMDA receptors (voltage dependent Mg^{2+} blockade and association

with Ca^{2+} channels) seem to represent the key aspects of LTP operation which underlie associativity and trigger long-lasting change. NMDA antagonists are, therefore, useful tools to investigate the mechanism and the physiological role of LTP.

1.3.3 LTP and memory

(a) Physiological properties of LTP relevant to a possible role in learning and memory

Several characteristics of LTP as follows suggest that it is an attractive candidate for an information storage system in the brain (Teyler and Discenna, 1984; McNaughton and Morris, 1987a ; Morris et al., 1989).

(1) Rapid onset: LTP can be induced by brief (tens of milliseconds order) stimulation of an afferent input (usually at 100 to 400 Hz) and develops over a time-course of about a minute. This observation accords with the fact that memories are rapdly formed by an event takes place only once.

(2) Long duration: A decay time-course of hours in acute *in vitro* preparations, and, in chronic *in vivo* LTP experiments, of greater than a day or a month, has been reported. This longer decay time course of LTP is reported to correlate with the rate of forgetting of spatial information in an animal learning task.

(3) Input specificity: When a single group of presynaptic fibres is conditioned, LTP is generated only in the tetanized fibres and not in untetanized fibres, even though they terminate on the same postsynaptic neurons. This property could be significant to increase the information storage capacity of the brain and to permit a parallel distributed processing style of computation. Although the suggested existence of a diffuse retrograde messenger like NO or CO during the early phase of

LTP induction casts doubt on this 'input specificity' principle, Zuho et al. (1993) put forward a possible solution to this problem in their experiment on hippocampal slices . They found that only the pathway which received weak tetanic stimulation paired with NO or CO exhibited enhanced and long lasting (at least 1 hr) EPSP but not the pathway which received weak tetanus stimulation 5 min before application of NO or CO. Hence, long-term enhancement mediated by NO or CO may still be spatially restricted to the synapses which also receive an active signal.

(4)Associativity: So called associative LTP is observed if a weak tetanus is delivered with a strong input. LTP will then be seen on the synapses that receive both inputs. This property of LTP is highly suggestive of conditioning paradigms, such as Pavlovian conditioning, in which a neutral (i.e. weak) stimulus paired with a reinforcing (i.e. strong) stimulus evokes a response corresponding to a 'memory' of the strong stimulus.

(b) Evidence that LTP represents the physiological synaptic enhancement

Barnes (1979) has found that memory deficit in aged rats is correlated with a faster decay of LTP. This evidence suggests that changes in the ability to maintain LTP underlie a substantial fraction of age related impairment of memory function.

It is demonstrated that artificial saturation of inducible LTP (= saturation of the capacity for synaptic weight change) in the dentate gyrus caused learning deficit in the circular platform maze (McNaughton et. al. 1986) and in the water maze *(Castro et al.1989). The synaptic weight change associated with experimentally induced

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* The reliability of the results reported by Castro et al., (1989) is now equivocal. Five individual groups have failed to replicate the spatial learning deficit following LTP saturation in the water maze task (Cain et al., 1993; Jeffery and Morris, 1993; Korol et al. 1993; McNamara et al., 1993; Sutherland et al., 1993; introductory commentary is given by Bliss and Richter-Levin in the same issue 1993). According to their comments, one possible explanation for this inconsistency is that it is not practically possible to saturate all synapses in the dentate gyrus and the original study may detect false positive learning deficit.

LTP is suggested to be equivalent to the natural change postulated to occur during memory formation.

(c) Experimental implications of LTP in learning and memory

Important behavioural evidence linking hippocampal LTP to learning and memory was provided by a series of experiments using the water maze paradigm (Morris et al., 1986a; Morris, 1989; Davis et al., 1992). It was shown that intraventricular infusion of AP5 (Mixture of D and L isomers was used for the experiments reported in 1986. Pure D-isomer was used in the later experiments.) caused an impairment of spatial learning in the water maze which is known to be highly sensitive to hippocampal damage (Morris et al., 1982), leaving no effect on visual discrimination learning which is not. The same AP5 treatment also suppressed LTP *in vivo*. The L-isomer of AP5 did not produce any behavioural effects or LTP suppression. These results suggested that AP5 impaired learning by disrupting hippocampal LTP.

The striking point of the above experiments was that they correlated two different levels of concept - a neurobiological concept (synaptic plasticity) and psychological concepts (learning and memory). In another words, this difficult work was only achieved by the combination of rigorous neurophysiological analysis and behavioural analysis.

This achievement has encouraged many researchers to investigate disruptive effect of NMDA antagonists on animal learning. Competitive and non-competitive NMDA receptor antagonists have also been reported to disrupt learning in radial arm mazes (Danysz et al. 1988; Ward et al. 1990), passive avoidance tests (Danysz et al. 1988; Parada-Turska and Turkui 1990), aversively motivated Y-maze tests (Tang and Ho 1988) and T-mazes (Flood et al 1990) and delayed conditional discrimination task (Tan et al., 1989) as well as other studies using the water maze tasks (see Table 1-1). Although many studies have discussed the relationship

between the effect of an NMDA antagonist on LTP and on learning and memory, very few presented substantial evidence to suggest an involvement of LTP in each learning model. These kinds of investigations should provide the evidence suggesting not only that the NMDA receptor antagonist blocks the change of synaptic efficacy (neurophysiological evidence) but also that the drug actually blocks the fundamental learning process (psychological evidence). As we do not have the method to measure either concept directly, we should carefully analyse the alternative indirect evidence concerning the above two points. Therefore, it should first be checked if the drug impairs the artificial induction of synaptic enhancement (LTP) in the equivalent condition to that in which animals have the learning task. This point is so far straightforward. The second point is not very easy. It should be checked if the drug actually impairs learning by blocking memory acquisition but not by some other 'side effect'. The 'side effect' means disrupting a prerequisite for learning performance; for example, causing a sensory motor disturbance or a disturbance in motivation or attention which is essential to perform the maze task. As escape performance in the water maze is motivated by aversion to the water, the experiment is sensitive to the psychological modulation of aversion. Ways to check the second point are discussed in the next section.

Table 1-1 The effect of AP5 and MK-801 on learning performance in Morris Water Maze

Author(s)	rat	submerged platform (place navigation) training				visible platform training	another test	
		dose and route of drug administration	water temperature	acquisition training	transfer test			
Morris (1989)	Lister-hooded male	**AP5** 40mM ICV chronic	26±1 °C	+	+	visual discrimination test —	place-recall test —	LTP induction +
Whishaw and Auer (1989)	Long-Evans female	**MK-801** i.v. 0.1, 0.05mg/kg 3 hr prior to training x 1day	18 °C	—	— (drug +)	—	changing-place task +	
Halliwell and Morris (1987)	Lister-hooded male	**MK-801** 0.1 mg/kg i.p. 20 min prior to training x 5days	25~26°C (presumption)	+	—	N.T.	LTP induction —	
Robinson et al. (1989)	Long-Evans male	**MK-801** 0.05mg/kg s.c. 20 min prior to training x 3days +2days(pre-training+transfer test)	27 °C	+	+ (drug +/-)	—	place-recall test —	post training administration test —
McLamb et al. (1990)	Fisher-344 male	**MK-801** 0.05mg/kg s.c. 1 hr prior to training x 8days	28±2 °C	+	— (drug -)	N.T.	post training administration test —	motor activity and startle response test —
Heale and Harley (1990)	Long-Evans male	**MK-801** 0.07mg/kg i.p. ~30min prior to training x 2days	no description	+	+ (drug -)	N.T.	place-recall test (transfer test only) —	open-field activity test *increase in activity*

+ : Impair — : No significant effect

1.4 The Water Maze

1.4.1 'Place navigation'

The open field water maze task developed by Morris (Morris 1981, 1984) has been proven very useful for investigating spatial learning and memory in rodents.

It consist of a large circular pool filled with cool water made opaque with milk powder. Submerged just below the water surface somewhere in the pool is a platform onto which the rat can climb to emerge from cool water and escape from the necessity of swimming.

In the standard use of the Water Maze (WM), a submerged platform (= hidden or invisible platform) is maintained in a fixed position and a rat is placed into the pool then allowed to swim until it escapes to the platform. This trial is repeated from variety of starting positions near the pool wall (the angular relationship between the platform and the starting position is randomly changed). The efficiency of escape, which is represented by the decrease of latency or path length taken to find escape, improves across training and reaches an asymptotic level suggesting a limit of the animal's ability. Because there are no cues within the pool to guide the rat to the platform, most rats solve this maze by learning the spatial position of the platform relative to distal (i.e. extramaze) cues (Morris, 1984). At the same time, the rats also learn some skills in searching for the platform (e.g. acquiring a specific sequence of movements). However, these non-spatial strategies are less effective in the WM and are generally not considered to represent true spatial learning. The acquisition of knowledge of the submerged platform's location based on extramaze cues is called 'place (space) navigation' or 'place (spatial) learning' (Morris et al., 1982; McNamara and Skelton, 1993). The asymptote of escape performance

exhibited by the animals after considerable training is close to the average of the shortest possible escape (the average of escape latency or escape path taken by the straight line escape from various starting positions to the platform), while the asymptote of escape performance of rats which fail to acquire spatial memory is a much longer escape latency or escape path.

A typical example of specific impairment of place navigation is observed in the performance of hippocampal lesioned animals (Morris et al., 1982). Morris et al. (1982) showed that although the escape latency of hippocampal lesioned animals was decreased with the repetition of training trials, it was significantly longer than that of control rats and reached a poorer asymptote. This asymptote level seemed to be comparable to the performance of normal rats trained with a submerged platform whose position was changed randomly on every trial. In such training, normal rats have no chance to memorise the position of the platform and move around randomly to search for it. Therefore the poor asymptote of escape performance of the hippocampal lesion rats suggested that they cannot acquire knowledge about the position of the submerged platform. This dissociation between the acquisition of spatial memory and the acquisition of non-spatial strategy is based on the principle of this task: the 'place navigation' is the best strategy to find the hidden platform.

The above advantage ruled by the principle of the task is one of the great strength of this learning model since many other learning tasks have trouble in distingishing between spatial and non-spatial components of learning. For example, in the radial arm maze task, not only the acquisition of spatial memory but also acquisition of non-spatial strategy (e.g. circuit strategy, particular angle movement strategy) achieves best performance. Thus it is basically hard to distinguish the improvement of performance due to the acquisition of spatial memory from that due to non-spatial skill in that maze task.

At the same time, the place navigation task in the WM has also practical advantages as follows:

(1) Intramaze cues such as odour trails are easily obviated.

(2) No pretraining is required and acquisition is quite rapid, allowing for large numbers of animals/treatments to be assessed in a short period of time.

(3) It has several measures of performance (e.g. escape latency, path length and path directionality) that are quantitative descriptions rather than highly abstract measures of observed behaviour.

In addition to the advantages of the place navigation task alone, the WM system as a whole provides a variety of reference procedures which are useful to examine side effects of experimental manipulations (drug administration, lesion surgery) on the performance of animals in the place navigation task. Some procedures are suitable to confirm whether the acquisition of place navigation is intact or not. Some are introduced to monitor disturbances in sensory-motor ability or motivation or attention. Because the reference procedures use the same animals and apparatus, and can be carried out without significant modification to the incentive or sensorimotor requirements of the task, they can be compared with the place navigation procedure directly.

Among these reference procedures, the transfer test, visible platform training, visual discrimination and place recall test are discussed in the following section.

1.4.2 Reference procedures

(a) Transfer Test

This is useful to detect the establishment of spatial memory in the animal and dissociate spatial and non-spatial strategies (Morris, 1981; 1984). This test is usually

carried out after the performance of rats in place navigation training reaches the asymptotic level. In this test, the escape platform is removed and a rat permitted to swim freely about the pool for a limited period (e.g. 60 sec). The use of a place navigation strategy is inferred if the rat spends more time in the quadrant that previously contained the platform or cross over its position more often than equivalent positions in the other three quadrants. This bias is quantified by the time spent or path length traversed in the target quadrant against the other quadrants and/or the number of crossing over the exact surface area of the platform. If the rats took non-spatial strategies, the swimming paths are not biased towards a particular quadrant or platform location; for example it may involve large circles at the appropriate distance from the pool wall, or be directed towards or away from a single cue (such as the experimenter). Morris et al. (1982) confirmed that the hippocampal lesion animals did not acquire any knowledge about the location of the platform during place navigation training by this transfer test. As this procedure represents the retrieval process of memory, it is also possible to test the drug's effect on the retrieval process specifically if the drug is administered only before this transfer test.

(b) Cue navigation training (Visible Platform Training)

In this procedure, the visible platform which protrudes above the water surface is utilised as the escape area in the pool in place of the hidden platform submerged under water (Morris, 1981; 1984). The rat is only required to learn to swim towards the visible object (cue) and escape onto it. The position of the visible platform and the starting position are changed from trial to trial in order to prevent the use of a spatial strategy. This reference procedure is useful to rule out the sensory motor and/or motivational impairment of the drugs or brain lesion. Morris et al. (1982) suggested the specificity of the effect of hippocampal lesion to the place navigation by the effective performance of the lesioned animals in this cue navigation training.

(c) Visual Discrimination Learning

In this task, two visible platforms which are painted in clearly different patterns from each other are present in the pool (Morris, 1984; Morris et al., 1986b). One of the platform is rigid and offers secure escape from the water. The other is floating such that when a rat attempts to get onto the platform, it immediately falls back into the water. Following repeated trials, a rat learns to swim straight to the rigid platform. The start position and location of the two platforms are changed from trial to trial. The pool is usually surrounded by curtains to minimise the influence of extramaze cues and the improvement in performance is expressed by the reduction of errors (i.e. occasions that the rat tries to climb onto the floating platform). The main purpose of this task is to dissociate the effect on spatial learning which is sensitive to the hippocampal damage from the effect on another type of learning which is found to be unaffected by hippocampal lesions. This test is also useful to check whether the effect of drug or surgery on learning is associated with sensory motor ability, motivation or a reinforcement process.

(d) Place Recall Test

This is place navigation training and/or transfer test given to the drug treated or lesioned rats that were already trained in the place navigation procedure prior to drug treatment or lesioning (Morris, 1989; MacNamara and Skelton, 1993). It may reveal impairment to almost all of the process required for proper performance of the task (sensory, motor, motivation, memory retrieval, spatial information processing) except for the process required for acquisition of spatial memory.

The above tests are only a few examples of control procedures which are available in the WM experiments. As this system has great flexibility, further modification and a variety of procedures is possible.

1.4.3 Some problems in the water maze experiments

Although the WM learning task is a powerful system to investigate brain function and drug effects on spatial learning, there are several points that we have to be careful of in analysing the animal performance in this system.

(a) Transfer test

Although the swimming bias during the transfer test is a good index of spatial memory of animals, it may not always precisely express their memory. Some of the normal rats acquire the knowledge about the location of platform quickly and learn to swim directly toward the platform in the early phase of place navigation training (late trials on the first day or early trials of the 2nd day). However, even these 'quick learners' show a significant swimming bias in the transfer test only after considerable repetition of training trials. If these quick learners are given a transfer test following good performances but insufficient training, they easily give up their attempt to recall their memory after short time of appropriate search and start to look round only for other places at which escape during the rest of the test period. This suggests that the biased swimming behaviour of rats in the transfer test requires not only the acquisition of spatial memory, but also the generation of confidence in, or persistency of, memory. In another words, an animal has a conflict between two types of strategy: the place navigation strategy and random search strategy, and the choice can be shifted by situations (repeated failure to find the platform) or psychological states during training or test trials. Therefore, if a drug just delays the acquisition of persistency or confidence, or, if it reduces the level of persistency or confidence, it may reduce the acquisition of swimming bias. The effect of anxiolytic benzodiazepine, chlordiazepoxide on place navigation learning reported by McNaughton and Morris (1987b) seems to show such an effect. In the transfer test,

they observed that "the chlordiazepoxide rat swims, initially, towards the correct annulus, but then he shows no tendency to stay in its immediate vicinity or to rotate in the water at the correct location". As this place/random conflict during test trials sometimes impedes an analysis of spatial memory of animals, some attempts to clear this problem have been reported.

Spooner et al. (1994) designed an "Atlantis platform" system in which a collapsible cantilevered platform is raised near to the water surface only if a rat dwells within a defined area for a set period of time in order to exclude the chance for a rat to find the platform by random search. As normal rats were forced to use only a place navigation strategy during training in this system, they showed a remarkably more focused search at the correct target location during the transfer test.

Another attempt to cope with place/random problems was reported by Whishaw et al. (1995). They analysed the swim path just at the beginning of the transfer test and rats were quickly removed from the water as soon as they swam out of the correct quadrant. The digitized swim path from start position to the point at which a rat came closest to the platform's position was interpolated by their own way to accommodate the data to averaging and statistical analysis. They also analysed locations at which rats changed their swimming direction after their swimming course had been set toward the platform. The turning points were characterized by a sharp deceleration in swim speed and an obvious body turn. This turning behaviour has been regarded as an attempt for the rats to look for the platform closely. In any case, the validity of their attempt may have to be examined further.

(b) Cue navigation training

Normal performance in cue navigation training may not always guarantee a normal level of escape motivation of test animals. There could be a difference in the

extent in anxiety or motivation between a task requiring uncertain escape (place navigation) and a task requiring swimming to a conspicuous secure object or, respectively, a difference between escape to a submerged platform and escape onto a platform which protrudes above the water and enable rats to get out of the water completely.

(c) Conceptual problems

I would like to add a brief comment on the above discussion from another point of view. As this thesis tries to discuss the LTP as a memory storage mechanism, a "memory" in animals simply means a storage of sensory information. However, I am not sure whether it is possible to distinguish a simple information storage process from various processes accompanying memory utilisation or mobilisation mechanisms clearly, because we can only detect an animal's memory through their behaviour. We can take a standpoint that memory process includes whole system to utilise or materiarise stored information. In this point of view, a deficit in the transfer test directly means a deficit in memory system. On the other hand, if we define 'memory' in narrow sense as a storage of sensory informations, deficit in transfer test should be caused by not only defect in memory storage but also any other kind of defects for example motivational problems or inhibition in another processes of learning such as consolidation, retrieval or expression of stored information. This standpoint requires the careful discussion whether the poor learning performance is simply due to inability of memory acquisition.

The combination of reference tests and close observation of animal behaviour may be helpful to investigate the actual mechanism of learning impairment induced by experimental manipulations.

1.4.4 Role of NMDA receptor activation in learning and memory in the water maze

Several groups have reported the effect of MK-801 on the learning performance of rats in the water maze (Halliwell and Morris, 1987; Whishaw and Auer, 1989; Robinson et al., 1989; McLamb et al., 1990; Heale and Harley, 1990). Although MK-801 is expected to block physiological activation of the NMDA receptor like AP5, the effect of MK-801 on learning seems to be different from that of AP5. Table 1-1 (in page 17) summarised the experimental conditions and results of these reports in comparison with the result of the AP5 experiment reported by Morris (1989).

(a) The effect of AP5 on learning in the water maze

In the experiment of Morris (1989), the impairment of place navigation caused by D, L-AP5 was demonstrated by two different measures of performance. The D,L-AP5 group showed a significant longer escape latency than controls during place navigation training (this is also expressed as a longer path length) and a poor swimming bias to the training quadrant during the transfer test. The transfer test was carried out after 5 days training (3 trials/day) because, by that stage, the performance of both control and AP5 animals reached asymptote level. The specificity of the drug's effect as an NMDA receptor antagonist was proved by the lack of impairment by L-AP5. A visual discrimination test was carried out to check the possibility that the impairment of spatial learning was caused by the impairment of motivation or reinforcement to escape to the platform or by the sensory motor disturbance. It was also checked whether non-spatial pretraining had an effect on place navigation. The pretraining reduced sensory motor disturbance during place navigation training, but had no effect on the drug-induced learning impairment. It suggested that, although some aspects of a sensory motor disturbance is induced by

AP5, it was probably not the main cause of spatial learning impairment. The lack of effect of AP5 on place-recall test suggested that AP5 did not impair the retrieval process of memory. It also reinforced the idea that the learning impairment caused by AP5 had nothing to do with the effect on motivation, or sensory motor ability. Finally, it was demonstrated that infusion of the same concentration of AP5 by the same route (i.c.v.) as that used in the WM experiments caused a total blockade of LTP *in vivo* but had no effect on normal fast synaptic transmission in the hippocampus. It was proposed that AP5 impaired place navigation learning by blocking synaptic plasticity in hippocampus. This proposal was reinforced by the dose response study showing a highly significant correlation between AP5 content in hippocampus and both percentage LTP and escape latency (Morris et al., 1990a; Davis et al., 1992).

(b) The effect of MK-801 on learning in the water maze

In contrast to AP5, the behavioural specificity of the effect of MK-801 on learning in the WM has been poorly characterised.

Among the WM studies examining the effect of MK-801, the experiment reported by Whishaw and Auer (1989) is hard to draw any conclusion because it was carried out under exceptional conditions: i) The water temperature was much lower than in other reports (18°C). ii) The rats received intensive pretraining (16 trials a day for 10 days) before just one day training with drug administration. iii) there was a 3 hr interval between drug administration and the commencement of training. These conditions may decrease the sensitivity of learning task to detect any effect of the drug.

Other experiments (Halliwell and Morris, 1987; Robinson et al., 1989; McLamb et al., 1990; Heale and Harley, 1990) showed similar results in the place navigation training. All four reported MK-801 (0.05 ~ 0.1 mg/kg) caused an

impairment in the rate of acquisition which was demonstrated by an increase in latency or path length taken to locate the platform during place navigation training. The cue navigation training and place recall test reported by Robinson et al. (1989), and the motor activity and startle response test reported by McLamb et al.(1990), showed that MK-801 at the dose of 0.05 mg/kg had no effect on sensory motor function or memory retrieval. Post training administration of MK-801 (Robinson et al., 1989; McLamb et al., 1990) revealed that MK-801 had little effect on memory consolidation. Therefore, the significantly poor performance during place navigation training induced by the 0.05 mg/kg MK-801 is suggested to be due to disturbances in the memory acquisition process.

The reports differed, however, with respect to the results of a transfer test. While Robinson et al.(1989) and Heale and Harley (1990) reported that MK-801 impaired the acquisition of spatial memory resulting in a poor performance in the transfer test. Halliwell and Morris (1987), and McLamb et al. (1990) reported that MK-801 had no effect on performance during transfer tests. This suggests that it is *not* impossible for rats which received 0.1 mg/kg or lower dose of MK-801 to memorise the location of the hidden platform. Halliwell and Morris also reported that MK-801 did not block LTP at the same dose. Although the effect of MK-801 on the performance during transfer test looks different from that of AP5, the result of the experiment using MK-801 was consistent with the result of the experiment using AP5 in showing that the drug does not block acquisition of spatial memory at a concentration of drug that does not block LTP.

It should be noted that in the experiments which demonstrated poor performance of the MK-801 group in the transfer test, animals were trained for short periods (Robinsons: 3 days, Heale and Harley: 2 days) while the experiments which demonstrated no inhibitory effect on memory, animals were trained for longer periods (Halliwell and Morris: 5 days, McLamb: 8 days). The poor performance of

MK-801 groups in the transfer test observed by Robinson et al and Heale and Harley and during training observed by all four groups could be due to a delay of acquisition rather than a complete block of acquisition of memory about the platform location

The result of LTP experiments in urethane anaesthetised rats by Halliwell and Morris (1987) (in which i.p. injection of 1 mg/kg MK-801 20 min prior to the tetanus stimulation did not block LTP) seems to be consistent with the results reported by Abraham and Mason (1988). They showed that 0.1 mg/kg or 0.5 mg/kg of MK-801 did not block the induction of LTP of dentate EPSPs in urethane anaesthetised rat if the tetanus stimulation was applied 30 min or 150 min after i.p. injection. In addition, 1 mg/kg of MK-801 given 30 min prior to the tetanus stimulation still did not block LTP induction, but when given 150 min prior to the tetanus stimulation, it did block LTP. Morimoto et al. (1991) have shown that LTP of EPSPs induced in the dentate gyrus of anaesthetised rat was blocked by the i.p. injection of 1 or 2 mg /kg MK-801 given 120 min prior to the tetanus stimulation. These results suggest that all four WM experiments in Table 1-1 were carried out under conditions in which MK-801 does not block LTP in the dentate gyrus.

A report by Gilbert and Mack (1990) claimed that 0.1 and 1.0 mg/ kg of MK-801 suppresses the LTP if the animal is not anaesthetized. However, what they called LTP was the LTP of population spike which is not a real LTP in the strict sense (* see further explanation below). Therefore their result may not be strong counter evidence to the claim made above. As they failed to induce LTP of the EPSP (because of an unstable EPSP in unanaesthetised animals) it is difficult to accept that their experiment is reliable.

Since AP5 impaired learning at the same dose range as it blocked LTP in anaesthetised rat, at least in neurophysiological terms, the above water maze

experiments using MK-801 were not carried out under the comparable conditions to those in which AP5 induced learning impairment.

To sum up, the effect of MK-801 on the learning performance of rat in the WM seems to be different from that of AP5 in the following points.

	AP5	MK-801
Escape Latency	significantly increased at dose exactly the same as it blocks LTP	significantly increased at a dose that has no effect on LTP
performance in Transfer Test	impaired	no effect after 5 or 8 days training

synaptic efficacy (Teyler and DiScenna, 1987), and the slope of the population EPSP (see Fig.4-1 in Chapter 4) is the authorized parameter for monitoring synaptic efficacy (Lømo, 1971). Sometimes LTP is demonstrated by the increase of population spike amplitude because potentiation of EPSP increases the probability of cell firing. However, the potent* Strictly speaking, LTP is the long lasting enhancement of iation of population spike is disproportionally greater than potentiation of the EPSP because the relation between EPSP and population spike (input-output curve) is shifted after the induction of LTP (Bliss and Lømo, 1973; Abraham et al., 1985). Therefore the potentiation of population spike involves some mechanisms independent of enhancement of synaptic efficacy and has not been systematically investigated at the intracellular level. If MK-801 suppressed the potentiation of population spike, this effect would not necessarily be mediated by the suppression of LTP. Abraham and Mason (1988) reported a significant decrease in amplitude of the population spike after injection of 0.5 mg/kg MK-801 at which no significant effect on basal EPSP or LTP was observed. They suggested that the effect on LTP and effect on population spike of MK-801 were unrelated.

1.4.5 Hippocampus and spatial learning

As is discussed so far, the hippocampus is an important site at which NMDA receptor antagonists exhibit their effects and at which the induction of LTP and some processes of spatial learning take place. However, I have avoided discussing how the hippocampus is involved in learning and memory. This is too great a subject for this thesis, to go into too much detaile of each incompatible theories (e.g. spatial map theory and working memory theory) However, the LTP hypothsis is compatible with either theory about hippocampus function. I will mainly discuss one of the theories of hippocampal function, the spatial map theory which is useful to explain that hippocampus dependent processes are specifically involved in the place navigation learning in the water maze.

First of all, it is important to point out that the term "hippocampus" is used to refer to "the pyramidal cell fields of the hippocampus proper (CA1-CA3) together with the hilar and granule cells in the dentate gyrus" (Jarrard, 1993) throughout this thesis. The word "hippocampal system" is used to refer to hippocampus with the major afferent and efferent projections.

Among the vast variety of theories about hippocampal function, "spatial map"(or "cognitive map") theory postulated by O'Keefe and Nadel (1978; 1979) is the most prominent and the best basis for the discussion about the place navigation in the water maze. According to this theory, rats in the water maze learn the spatial map of environment representing the goal object and their own location relative to the extramaze cue. This hypothesis well explains the following features of place navigation learning in the water maze:

(1) Rats can rapidly learn to find an object (platform) that they cannot see, hear, or smell.

(2) Rats show good directionality in the path (straight escape path) taken from any start position.

(3) The place navigation is a learning dependent on the distal cues and is dissociated from the learning dependent on proximal cues (cue navigation) by the sensitivity to hippocampal damage.

(4) When rats are required to approach from a novel starting location, they can adapt to find the platform with no measurable increase in latency (Morris, 1981).

(5) Rats can rapidly adapt to a new escape location when the fixed position of the platform is changed (Morris, 1981).

Point (3) represents and justifies discrimination of two classes of learning: 'locale' and 'taxon', assumed in the "spatial map" theory. In the 'taxon' system, rats guide themselves by approaching or leaving a particular object (proximal cue) or by taking a specific sequence of movements. This system is suggested to be located outside the hippocampus. In the 'locale' system, rats identify a place using spatial configuration of sets of stimuli (extramaze cues). This understanding of a place is a spatial map and is supposed to be organized in the hippocampus.

The difference between taxon and locale system is not just a difference of the utilised cue (intramaze versus extramaze cue) but a difference in the place representation with the cues. The performances of rats with damage to the hippocampal system reported by Eichenbaum et al. (1990) is a good example which emphasises this difference. They trained the animals to escape to the platform from the same starting location to encourage rats to associate the extramaze cues with a particular swim trajectory and with the place of escape. The escape platform located in the fixed position was initially visible but the visibility was gradually faded out. Both normal rats and rats with Fimbria-Fornix lesion (FX rats) acquired this task rapidly and eventually both groups could locate the submerged platform (the

accurate navigation of both groups was confirmed by the proper swimming bias in the transfer test). However, the FX rats, unlike normal rats, could not escape properly when the cues just behind the goal were moved or starting position was changed. In short, both normal and FX rats eventually learned to locate the hidden platform relying solely on extramaze cues but their understanding about the place showed an apparent difference in the flexibility when a minor spatial modification was introduced. These results suggests that the two groups used completely different strategies. As is discussed further in the following section, the flexible adaptation of the normal rats to the spatial modification in the task suggests that they employ the extramaze cues as locale cues (references for the map) while the poor flexibility of FX rats to a novel situation suggests that they employ the extramaze cues as taxon cues. (However, Eichenbaum et al. did not discuss this point clearly.)

The behavioural flexibility, demonstrated by experiments (4) or (5), is a particular feature of spatial learning predicted by the spatial map theory. In the case (4), changing the starting position does not modify the spatial relationship in the map and in the case (5), moving platform location only requires a change to the map entry denoting the position of the platform. Therefore, rats can flexibly apply their spatial memory (map) to locate a goal in novel situations, provided the environment remains constant.

Whishaw et al. (1995) recently reported a study which is similar to that of Eichenbaum et al. (1990) and challenged the dependence of spatial map on hippocampal function. The main difference in experimental procedure is that they trained rats to swim from each of the four starting positions instead of a single fixed position during training with a visible platform and following training with a hidden platform located in the same place. The performance of FX rats was basically as accurate as that of control rats on the trials of visible platform training, hidden platform training and transfer test. They assumed that the impairment of FX rats is

not in learning the location of the platform in relation to extramaze cues ("knowing where") but in some process of motoric control ("getting there"). As FX rat eventually locate platform without intramaze cue, they explained that the damage in the hippocampal system did not impair acquisition of spatial map. Because the poor performance of FX rats was improved by the experience to swim directly to the goal, they suggested that the process of the acquisition of motoric refinement was damaged in the FX rats. However, utilising extramaze cue to locate a particular place is not necessarily a spatial map learning as is discussed above. It may be possible for rats to achieve the accurate performance by the establishment of independent approaching process to the target from the four starting points with the aid of extra maze cues (utilisation of extramaze cue as 'taxon cue'). Of course, it may be much easier for normal rats to utilise extramaze cues as 'locale cue' than 'taxon cue'. Only the combination of the inability to acquire the 'locale' navigation with the special training (training from the same start position or training with fading out platform) is supposed to enable rats to utilise the extramaze cues as the 'taxon cue' to guide themselves to the platform. What Whishaw et al. called motoric refinement process could be this particular process. Therefore, the results of this experiment cannot deny the dependence of the acquisition of spatial map on the normal hioppocamal function.

Another example of speculative commentary on the spatial map theory is recently made by Amsel (1993). He claims that the spatial learning is a simple discrimination learning. The escape behaviour is an accumulating go/no-go decision and the position of the platform should be the focal point of the weight of go decision. The hippocampus is a centre for conflict between approaching and avoiding some distal (extramaze) cues. Therefore the association between escape behaviour and extramaze cue depends on the hippocampal function. This hippocampal dependent conflicting (namely, vicarious trial and error) theory seems

to explain the hippocampal function in both spatial and nonspatial learning uniformly. However, it is not clear the difference between a navigation with an accumulating go/no-go decision and snap shot like monitoring of position with extramaze cue in the Eichenbaum's FX rats. Further experimental evidences may be necessary to show that the vicarious trial and error process specificcaly take place in the hippocampus.

While the spatial map theory discusses a specific type of information that is processed by the hippocampus, a number of alternative hypotheses were proposed aiming at more abstract characterisation of hippocampal functions. Among them, working memory theory (Olton et al., 1979) is a well-known opponent in debates. They proposed that the hippocampus is selectively involved in behaviours that require working memory irrespective of the type of information (spatial or nonspatial) which is processed. The working memory holds information pertinent to only a single-trial of an experimental procedure. The reference memory holds information pertinent to several trials. Their radial arm maze studies revealed that damage to the hippocampal system produced a similar deficit in both spatial and nonspatial test procedure. In contrast, the lesions exclusively impaired the performance requiring working memory but no effect was observed on the bases of the reference memory.

In opposition to this claim, many studies have been reported to show that damage to the hippocampal system impairs spatial reference memory but not nonspatial reference memory (for example, Morris et al., 1986b; Jarrard, 1993). I do not intend discussing this controversy further. In my opinion, the structure of animal memory is task dependent. The identical classification of memory cannot be applyed to the different learning tasks. It may not be possible to discuss 'reference memory' in radial arm maze and that in water maze uniformly. The 'working

memory' system utilised in radial arm maze may not be utilised in water maze. The important point seems to be that spatial map system or spatial navigation is a

characteristic and interesting system in the animals to process and memorise sensory information.

In this thesis, the drug's effect is tested on the place navigation task. The dependence of learning on hippocampal function will be confirmed by hippocampal lesion experiments.

1.5 Other behavioural effects of NMDA antagonists

1.5.1 NMDA receptor and conventional synaptic transmission in the brain

It is generally accepted that NMDA receptors have a special role in plasticity but contribute very little to normal synaptic transmission on pyramidal cells in hippocampus (Collingridge et al., 1983). However in other part of brain, there is some evidence for the involvement of NMDA receptor mediated synaptic transmission in the normal physiological state. For example, AP5 has been reported to block low-frequency transmission in slices of rat somatosensory cortex (Thomson et al. 1985). Sensory input in the thalamus and thalamocortical, cortico-rubral and thalamo-midbrain pathways in rats have also been found to be mediated by NMDA receptors (Headley and Grillner 1990). Esguerra et al. (1992) suggested NMDA receptors participate in fast synaptic events (less than 10 msec) underlying conventional retinogeniculate transmission in the ferret brain.

Therefore, administration of NMDA antagonists may produce some effects in animals due to change in spontaneous neural activity outside the hippocampus. In fact NMDA receptor antagonists have been reported to evoke behavioural responses, discriminative stimulus effect, decreased response to auditory stimulus, EEG desynchronization indicating enhanced arousal and weak anxiolytic effects (Wroblewski and Danysz, 1989).

1.5.2 Ataxia and stimulant effects on behaviour

PCP like non-competitive NMDA antagonists (e.g. MK-801, PCP, ketamine), as well as competitive antagonists (e.g. AP5, AP7, CPP, CGS19755) produce a complex behaviour syndrome, including increased locomotion, sniffing, head weaving, body rolls and falling, which progresses to ataxia with increasing dose in mice (Tricklebank et al., 1989; Koek and Colpaert, 1990; Liljequest et al., 1991). In rats, PCP and MK-801 were reported to produce similar hyper activity, stereotyped behaviour and ataxia (Koek et al. 1989, Hiramats et al. 1989, Tiedtke et al. 1990, Kretschmer et al. 1992). The following evidence suggests that the inhibitory effect on NMDA receptor mediated neurotransmission may be directly involved in the behavioural effects of NMDA antagonists:

1) Competitive and non-competitive NMDA antagonists induce qualitatively similar behavioural effects in spite of the lack of similarity in their chemical structure (Schmidt, 1986; Tricklebank et al., 1898; Koek and Colpaert, 1990)

2) The potencies for competitive and non-competitive NMDA antagonists to produce locomotion and falling correlate ($r = 0.92$ $p < 0.01$) with their relative potencies to antagonize NMDA-induced convulsions (Koek and Colpaert, 1990).

3) PCP-type non-competitive NMDA antagonists, PCP, ketamine and MK-801, induced hyperlocomotion and falling in mice with a potency order (MK-801 > PCP > ketamine) similar to their relative affinities for the PCP receptor (Koek and Colpeart, 1990). Danysz et al. (1994) reported MK-801, PCP and ketamine induced hyperlocomotion, ataxia and stereotypy (head waving) in rats and correlated their potency of their capacity to inhibit [^{3}H]-MK-801 binding. This order has no relation with their affinity to the sigma binding site (Wong et al., 1988), or to the dopamine uptake site (Vignon et al., 1988).

4) D-serine, a selective agonist at the strychnine-insensitive glycine binding site of the NMDA receptor complex, antagonized PCP and MK-801 induced stereotyped behaviour and ataxia (0.5 ~ 1.0 μmol i.c.v./rat) (Contreras, 1990).

5) D-alanine, but not L-alanine (10 - 100 μg/side i.c.v.) attenuated hyper locomotion induced by PCP (100 mg/kg) in the rat. This stereo selectivity agrees with the potency of these agents as agonists for the strychnine-insensitive glycine site (Tanii et al., 1991).

In the following section, recent more detailed neurochemical and behavioural research of NMDA receptor antagonists are discussed to get a clearer idea about whether the behavioural effects of MK-801 and another PCP-type antagonists are really exhibited though their inhibitory activity against NMDA receptor mediated neurotransmission and what kind of mechanisms are involved.

Though we have abundant information about the behavioural effects of PCP, the following discussion will be made mainly about MK-801 since PCP has high affinity for the sigma binding site (Ki = 0.5 ~ 3 μM) which could explain part of the behavioural effect of PCP (Largent et al., 1988; Contreras et al., 1986). MK-801 is a highly selective ligand for the PCP site of the NMDA receptor complex with little effect on the sigma recognition site or dopamine uptake site at a pharmacologically relevant concentration (Lodge and Johnson, 1990)

1.5.3 Involvement of catecholaminergic system

One of the earliest pharmacological reports of MK-801, that by Clineshmidt et al (1982b), suggested the participation of receptors for catecholamines in the stimulus effect of MK-801. (1) The behavioural syndrome caused by MK-801 was similar to the behaviour observed after administration of classic psychostimulants like amphetamine. (2) MK-801 (0.05 mg/kg p.o.) induced ipsilateral turning in rats with a unilateral nigrostriatal lesion produced by 6-hydroxydopamine. MK-801 induced turning is reduced by haloperidol or alpha-methyl-p-tyrosine and blocked by reserpine. (3) Stimulation of locomotor activity in mice produced by MK-801 (0.3 mg/kg p.o.) was abolished by reserpine and significantly antagonized by haloperidol.

Following this study, investigations about the involvement of dopaminergic and other catecholaninergic system in the behavioural effect of MK-801 have accumulated. As shown in Table 1-2, MK-801-induced hyper activity and stereotypy is reported to be inhibited by selective D_1 and D_2 dopamine receptor antagonists or D_1/D_2 mixed antagonist (Dall'Olio et al., 1992; Hoffman, 1992; Löscher and Hönack, 1992; Verma and Kulkani, 1992; Ouagazzal et al., 1993). These studies suggest that both D_1 and D_2 dopaminergic receptors may be involved in the behavioural effects of MK-801.

This suggestion seems to be supported by some biochemical data showing an increase of dopamine metabolites (DOPAC or HVA) in various brain regions following MK-801 administration. In these reports, MK-801 was demonstrated to increase dopamine turnover in striatum and some parts of cortex (Hiramats et al. 1989, 0.5 mg/kg i.p. ; Rao et al. 1990, 0.3~1 mg/kg i.p. ; Lösher et al. 1991, 0.3 mg/kg i.p.), in the nucleus accumbens (Lösher et al. 1991, 0.3 mg/kg i.p.) and in other parts of brain (Rao et al. 1990, 0.3~1 mg/kg i.p. ; Lösher et al. 1991, 0.3mg/kg i.p.) in rats. Liljequist et al. (1991) suggested that MK-801(0.2 mg/kg i.p.) increased

Table 1-2 Modulation of behavioural response to MK-801 by dopamine receptor antagonist

Author(s)	**animal**	MK-801	**Stimulated Behaviour**	**DA antagonists that suppressed MK-801 induced behaviour significantly**
Clineshmidt et al, (1982 b)	Mice female CF1	0.3 mg/kg p.o.	hyperlocomotion	Haloperidol : 0.125 mg/kg i.p.
Dall'Olio et al.	Rats male SD	0.25 mg/kg i.p.	hyperlocomotion	SCH23390 (D_1) : 0.005 mg/kg s.c. (-) Sulpiride (D_2) : 0.0025 mg/kg s.c.
Ouagazzal et al. (1993)	Rats male Wister	0.3 mg/kg i.p.	hyperlocomotion	SCH23390 (D_1) : 0.04mg/kg s.c. Raclopride (D_2) : 0.1 - 0.3mg/kg s.c
Hoffman (1992)	Rats male SD	0.1 mg/kg s.c.	hyperlocomotion stereotypic response	Haloperidol : 0.05 - 0.5 mg/kg s.c. Eticlopride (D_1) : 0.01 - 0.05 mg/kg s.c
Lösher and Hönack (1992)	Rats female Wister	0.1 mg/kg i.p.	hyperlocomotion head weaving	Haloperidol : 0.1 mg/kg i.p.
Verma and Kulkani (1992)	Mice either sex Albino	0.1 mg/kg	stereotypic response	Haloperidol : 0.5 mg/kg i.p. SCH23390 (D_1) : 0.1mg/kg i.p. Molindone (D_2) : 2.5mg/kg i.p.

the rate of dopamine metabolism in the striatum and limbic forebrain of mice. However, in some experiments, MK-801 did not increase DOPAC or HVA in the striatum (Gandolfi et al., 1990; Bubser et al., 1992) or in the nucleus accumbens (Rao et al., 1990) of rats. The inconsistencies of results between experiments could be explained by the variety in strain, sex and age of experimental animals and difference in the dosages of drugs, extraction timing and procedure for measuring metabolites of dopamine. Lösher et al. (1993) compared the behavioural and neurochemical effects of MK-801 and CGP37849 (competitive NMDA receptor antagonist) in the unified condition. MK-801 and CGP37849 increased dopamine turnover in selective brain regions at equipotent doses for inducing stimulus effects on behaviour. It is strongly suggested that blockade of NMDA receptors induces alterations in dopaminergic activity and amphetamine-like adverse effects.

Microdialysis experiments directly demonstrated an increase of extracellular concentration of dopamine in the nucleus accumbens and caudate after local application of MK-801 (10^{-6} and 10^{-5} M) to respective region of rat brain (Imperato, 1990) and in the prefrontal cortex after systemic application of MK-801 (0.4 mg/kg) to rats (Wedzony et al., 1993). NMDA receptor antagonists may reduce the activity of an inhibitory input to dopaminergic neurons and stimulate dopamine release which, in turn, is possibly responsible for the hyper locomotion and stereotypies.

However, other evidence suggests that the underlying mechanism of the behavioural effect of MK-801 may be more complicated than that of dopaminergic agents like amphetamine. Although MK-801 induced locomotor activity is attenuated by either D_1 or D_2 dopamine receptor antagonists, this sensitivity is less than that of d-amphetamine -induced locomotor stimulation (Ouagazzal et al., 1993). Consistent with this result, the increase in locomotor activity produced by MK-801 (2.5 ~ 5 μg) infused into nucleus accumbens is not blocked by intra-accumbens infusion of haloperidol at a dose (2.5 μg, 15 min prior to MK-801) sufficient to

block amphetamine-induced locomotor activity. In addition, Wedzony et al. (1993) found that the locomotor hyper activity induced by MK-801 (0.4 mg/kg) was not correlated with an alteration in the extracellular concentration of dopamine in the prefrontal cortex of rats. These authors suggest that the complexity of the behavioural effects of MK-801 may be partly explained by the involvement of a novel category of dopamine receptor subtypes and/or other monoaminergic transmitter systems in the brain.

Recent molecular-genetic studies have revealed the existence of additional members of the Dopamine receptor family in humans and rodents. The cDNA for receptor subtypes of D_1, D_2, D_3, D_4 and D_5 has been cloned from the human gene (Seeman and Van Tol, 1994). The D_4 receptor draws pharmacological attention as a possible target of clozapine which is an 'atypical' neuroleptic with less extrapyramidal motor side effects.

Tieditke et al. (1990) reported clozapine (5 mg/kg s.c.) potently antagonize MK-801-induced stereotypy in rat. Hoffman (1992) demonstrated that clozapine reduced MK-801-induced locomotor activity at the dose of 1.0 ~ 10.0 mg/kg (s.c.) and sniffing at the dose of 10.0 mg/kg (s.c.). There is a possibility that MK-801 activated neurotransmission is mediated by a D_4 like receptor subtype in rats.

The α_1-adrenenoceptor antagonist prazocine has been shown to attenuate MK-801 induced hyperlocomotion and head weaving in rats (Lösher and Hönack, 1992) and hyperlocomotion in mice (Clineschmidt, 1982b) while α_1- and α_2-adrenoceptor agonists were found to potentiate motor activity induced by MK-801 (Carlsson and Carlsson, 1990).

In addition to the effect of adrenergic agents, there is also evidence to suggest that serotonin receptors are involved in the behavioural alterations produced by MK-801. Lösher and Hönack (1992) reported that the 5-HT_{1A} receptor ligands, ipsapiron and NAN-190 (which are known to display antagonist-like properties in *in vivo*

models) inhibited hyperlocomotion and head weaving induced by MK-801 (0.1 mg/kg i.p.). This result was consistent with the neurochemical observation that MK-801 (0.3 mg/kg i.p.) increased 5-HT turnover in several brain regions (e.g. frontal cortex, amygdala, striatum, nucleus accumbens) (Lösher et al., 1991). Following these investigations, Lösher et al. (1993) confirmed that an increase in 5-HT turnover and behavioural effect (ataxia, hyperlocomotion, head weaving) were induced after administration of MK-801 (0.3 mg/kg i.p.) under identical experimental conditions.

Microdialysis experiments showed that MK-801 increases not only serotonin metabolism but also its release in some brain regions including striatum (Whitton et al., 1992).

1.5.4 Involvement of catecholamine independent systems

The involvement of catecholamine independent mechanisms in the behavioural effects of MK-801 was also suggested by the work of Carlsson and Carlsson (1989) which revealed that MK-801 (1 ~ 4 mg/kg i.p.) caused a pronounced and dose dependent increase in locomotion in catecholamine depleted mice (using reserpine and α-methyl-para-tyrosine) and that this hyperlocomotion was not attenuated by haloperidol (1 mg/kg) pretreatment. However, it should be noted that the dose of MK-801 in this experiment was about 10 times higher than the dose in the above mentioned experiments revealing catecholamine dependent effects of MK-801. Probably both mechanisms (catecholaminergic and non-catecholaminergic) may be involved in the behavioural effects of MK-801 and non-catecholaminergic mechanisms might become emphasized after administration of a higher dose of MK-801.

1.5.5 Differential effect between competitive and non-competitive NMDA antagonists

The behavioural studies which compare the effect of competitive and non-competitive NMDA receptor antagonists showed that the potency of the competitive antagonists to produce stimulatory effects was relatively weak (Tricklebank et al., 1989; Liljequist et al., 1991; Svensson et al., 1991; Kretschmer et al., 1992; Lösher et al., 1993). For example, Lösher et al. (1993) reported the dose of MK-801 (0.3 mg/kg i.p.) required to induce behavioural change was about 4 times higher than its anticonvulsant ED_{50} in rats, while the dose of CGP37849 a competitive NMDA antagonist (30 mg/kg i.p.) was about 15 times its anticonvulsant ED_{50}. Another competitive NMDA antagonist, CGP39551, exhibits much less hyperlocomotion and stereotyped behaviour than CGP37849, although both drugs have a similar potency in the anticonvulsant action in rodents. Willetts et al. (1990) calculated the ratio of ED_{50} for motor impairment to ED_{50} for NMDA-induced seizure or lethality of various NMDA antagonists. The index for PCP like antagonists was in the range of 0.1 ~ 1.3 while the index for competitive antagonists was in the range of 1.0 ~ 11.9.

In the induction of behavioural effects of competitive antagonists as well as PCP-type antagonists, the involvement of the dopaminergic and serotonergic mechanisms is suggested. In the above report of Löscher et al. (1993), it was demonstrated that MK-801 and CGP37849 increased dopamine and serotonin turnover in selective brain regions at behaviourally equipotent doses whereas CGP39551 was almost devoid of such effects. Consistently, the behavioural effects induced by competitive NMDA antagonists have been reported to be blocked by dopamine and serotonin antagonists (Imperato et al., 1990; Löscher and Hönack, 1991). Although some reports found CPP-ene and AP5 to enhance significant dopamine release in nucleus accumbens and striatum (Gruen et al., 1990; Imperato,

1990), some reports demonstrated that systemic or central administration of AP5, AP7, CPP, CPP-ene, CGP39551 and CGS19755 were without effect on dopamine or serotonin turnover (Kabuto et al., 1987; Rao et al., 1990; 1991; Svensson et al., 1991; Bubster et al., 1992). However, it seems likely that if competitive NMDA antagonists are administered at doses which induce comparable behavioural activities, they could induce a similar alteration in dopamine or serotonin turnover in specific brain regions to those of PCP type antagonists.

The reason for the lesser potencies of competitive NMDA antagonists in producing stimulant effects on behaviour and alteration in dopaminergic and serotonergic activity are not clear at present. Rao et al. (1990; 1991) have explained the marked difference between competitive and non-competitive NMDA receptor antagonists by the existence of binding sites for non-competitive NMDA receptor antagonist that are not coupled to the NMDA binding site. According to this hypothesis, NMDA receptor-uncoupled binding sites would be responsible for those effect of non-competitive NMDA receptor antagonists that cannot be reproduced by competitive NMDA receptor antagonists. The existence of several subtypes of NMDA receptor suggested by the molecular pharmacological study (discussed in the first section) might be a possible explanation for the existence of those different binding sites.

Apart from a difference in the receptor itself, an interaction with the factors surrounding the NMDA receptor (e.g. glutamate) may also explain the difference. Extracellular glutamate is known to be actively modulated by a glutamate uptake carrier (transporter), which exist in both neurons and glia and usually keeps glutamate below toxic levels. However, it sometimes operates backwards to increase glutamate levels in pathological conditions (e.g. ischaemia) (Nicholls and Attwell, 1990). Recently three high affinity, sodium dependent glutamate transporters of rats were cloned: GLT-1 (Pines et al., 1992), EAAC1 (Kanai and Hediger, 1992) and

GLAST (Storck et al., 1992). Immunohistochemical evidence showing a selective localization of each transporter subtype suggests that glutamate levels are modulated in a site specific manner in the rat CNS (Rothstein et al., 1994).

Speliotes et al., (1994) found an example which suggests that glutamate uptake can modulate the potency of an NMDA receptor antagonist. In an astrocyte-rich rat cortical culture, AP5 was more potent against NMDA-mediated toxicity than glutamate-mediated toxicity because glutamate is taken up on its transporter thus very high concentrations of glutamate are necessary to induce toxicity. AP5 is, however, equipotent against the two in astrocyte-poor cultures in which transporter is absent. In contrast, MK-801 was similar in potency against glutamate and NMDA in both astrocyte-rich and astrocyte-poor cultures. If there is a brain region at which the extracellular glutamate level is high, the potency of AP5 can be relatively weaker than that of MK-801 at that region. Wallace et al., (1992) suggested physiological changes in extracellular glutamate level modulate the binding of MK-801 in the brain. They demonstrated that the binding of i.v. administrated [^{3}H] MK-801 to ischaemic cortex and striatum is significantly enhanced relative to normal brain.

In addition to the glutamate trapping/releasing system, brain tissue also seems to have a system to trap drugs. Morris et al. (1990a) and Davis et al. (1992) reported that the whole- tissue level of AP5 is about 30-fold higher than the extracellular level (based on the microdialysis data) in hippocampus after chronic infusion of AP5. Perfusion of 20 mM ethylene-bis(oxyethylenenitrate)tetra-acetic acid (EGTA) in Ca^{2+} free artificial cerebrospinal fluid (aCSF) instead of normal aCSF caused masssive release of AP5 in the dialisate. These data suggest that a significant proportion of whole-tissue AP5 is trapped to the cellular membrane by a Ca^{2+}-dependent mechanism. Supposition of different trapping systems or different trapping affinity for competitive and non-competitive antagonists could also explain the different potency.

1.5.6 Summary

The behaviourally stimulant effects of non-competitive NMDA receptor antagonists are suggested to be mediated by the blockade of NMDA receptor mediated neurotransmission outside the hippocampus. These effects of non-competitive NMDA receptor antagonists are exhibited at a relatively lower dose than that at which they exhibit an inhibitory effect on epilepsy or LTP in the hippocampus, while competitive antagonists exhibit the behavioural effect at the same or a higher dose than that at which they block LTP or epilepsy. Therefore, the effect of MK-801 or FR115427 on learning is possibly dominated by "side effects". The dose response and the time course of the drug's effect on behaviour and LTP need to be carefully analysed to check if it is possible to distinguish these effects. This insight is a relevant aspect of the design of the experiments to be reported in this thesis.

1.6 Aims of the thesis

This thesis examines whether the effect of non-competitive NMDA receptor antagonists: MK-801 and FR115427 on spatial learning is predominately related to their stimulatory effect on behaviour or related to their inhibitory effect on LTP.

It is expected that this comparative analysis gives us some clue about how NMDA receptors are involved in the spatial learning process.

Chapter 2
Material and methods

2.1 Subjects

Male Lister-hooded rats (199 ~ 466g) from the breeding stock of the Department of Pharmacology University of Edinburgh, were used. The rats were maintained in the University's animal house which provides the environment with constant room temperature (about 25°C) and a light/dark cycle of 14:10 hours (lights on at 0800hr). The rats were housed in group of 4 or 5 with sawdust and filter paper for bedding. In the water maze experiments only, the rats were transferred to individual cages after the habituation trials as described below. Free access to food and water was allowed throughout. The animals was brought out from the animal house to the laboratory at the beginning of each day's experiment and they were brought back to the animal house or sacrificed at the end of the day's experiment. All experiments were carried out in the light phase (8 am - 10 pm).

2.2 Drugs

(a) FR115427 ((+)-1-methyl-1-phenyl-1, 2, 3, 4-tetrahydroisoquinoline hydrochloride)

FR115427 was synthesised in the New Drug Research Laboratories, Fujisawa Pharmaceutical Co. Ltd., Osaka Japan.

(b) MK-801 or Dizocilpine ((+)-5-methyl-10, 11-dihydro-5H-dibenzo [a, d] cyclohepten-5, 10-imine maleate)

MK-801 was obtained from Research Biochemicals Inc., U.S.A.

Other drugs were obtained from Sigma and were of the highest available purity.

(c) Urethane Carbamate

Urethane Carbamate was used as a non-recoverable anaesthetic during electrophysiological phase of experiments. Urethane carbamate was dissolved in saline at 500 mg/ml and given by single i.p. injection (1.5 g/kg) at the start of the surgery prior to electrophysiology.

(d) Tribromoethanol (Avertin)

Tribromoethanol was used as a recoverable anaesthetic during surgery for making hippocampal lesions. A stock concentration (2,2,2,-tribromoethanol 1.6 g/ml in absolute ethanol) was kept at 4°C, in a dark container to avoid light degradation. A dilution of 1 in 55 was made in saline, 12 hours prior to surgery. The initial injection (i.p.) dose was 10 ml/kg body weight (tribromoethanol 0.29 g/kg), supplemented by a 0.5 ml injection as required throughout the surgery.

(e) Ibotenic acid (α-amino-3-hydroxy-5-isoxazoleacetic acid)

Ibotenic acid was used as neurotoxin to remove the cells in the hippocampus. The stock solution was made by dissolving ibotenic acid in phosphate-buffered saline at a concentration of 10 μg/μl and a pH of 7.4. Aliquots of approximately 50 μl of solution were stored in a freezer and thawed at room temperature for use.

2.3 Water Maze Experiments

2.3.1 Equipment

The spatial learning experiments were carried out in an open field water maze, developed by Morris (1981,1984). The maze consisted of a large circular tank of opaque water and a small escape platform.

(a) Environment

A cue environment was created in the rectangular (4.2m × 4.6m) room (Fig. 2-1A). The wall were painted white. A black curtain was hung from the ceiling on the arbitrarily designated West wall. Two metal frame stands (190 cm height, 55 cm width) were placed in the vicinity of the North East and West sides of the pool as conspicuous cues. The top of the frame on the West side was covered with a black plastic bag. Small posters were put on the East side and South side walls. A cabinet was placed near the south west corner. The South side wall was a partition between the pool room and the computer operating room with a window and a door way which may have also functioned as cues.

(b) The pool

The pool was 2.0m in diameter and 0.6m off the floor. The structure was made of glass fibre and coated with white gel coat. The pool was plumbed into the water system and could be automatically filled and drained daily. Though the water level in the tank (0.4 m high) and water temperature (25°C) was roughly set by an automatic operating system, the exact water level (1.5 cm above the height of the hidden platform or 1.5 cm below the top of the visible platform) and water temperature (25.0 or 27.0°C) were adjusted manually just before the experiment. The water was made

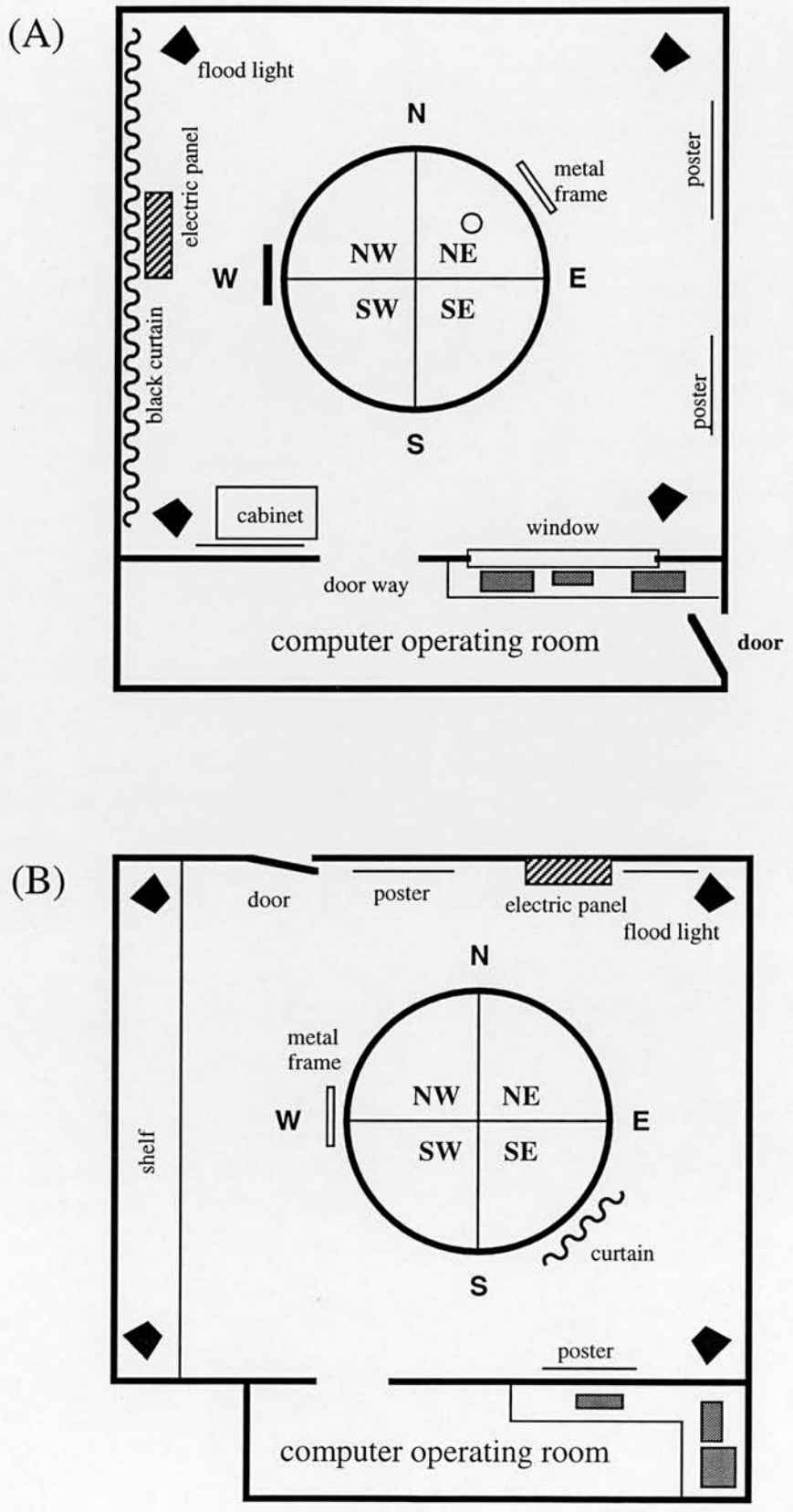


Fig. 2-1 Top view of the room for water maze experiments (A) and Open Field Activity tests (B)

opaque by adding fresh milk or milk powder. The water surface was divided into four "quadrants" as shown in Fig. 2-1 (NW, SW, SE and NE) for the convenience of later analysis.

The escape platforms were made from sections of Plexiglas tubing (10 cm in diameter) filled with stones and sealed at the top to provide the platform for rats and the bottom to form a base board. Two types of platform were used. One was the submerged (hidden) platform whose height was arranged to be about 1 cm below the water level and whose top was painted grayish green to match the water colour. The other was the visible platform which was arranged to be about 2 cm taller than the submerged platform to protrude just above the water surface and painted with black and white stripes. The hidden or visible platform was usually placed in the centre of one of the four quadrants during training.

(c) Tracking system

The swimming behaviour of the animals was monitored using a video tracking system. A camera was fixed above the pool in a position where the entire water surface area could be viewed (Fig. 2-2). Using 4×500 watt halogen flood lights angled toward the corners of the ceiling created sufficient diffuse lighting to allow image analyzer (HVS Ltd. model VP110) to detect the black head of a swimming rat against the pale colour water of the pool. The image analyzer sampled the position of the rat's head at the rate of 10 Hz and relayed the x and y coordinate to an Archimedes microcomputer where the data coordinates were stored on diskettes and used to calculate several measures of performance as described below.

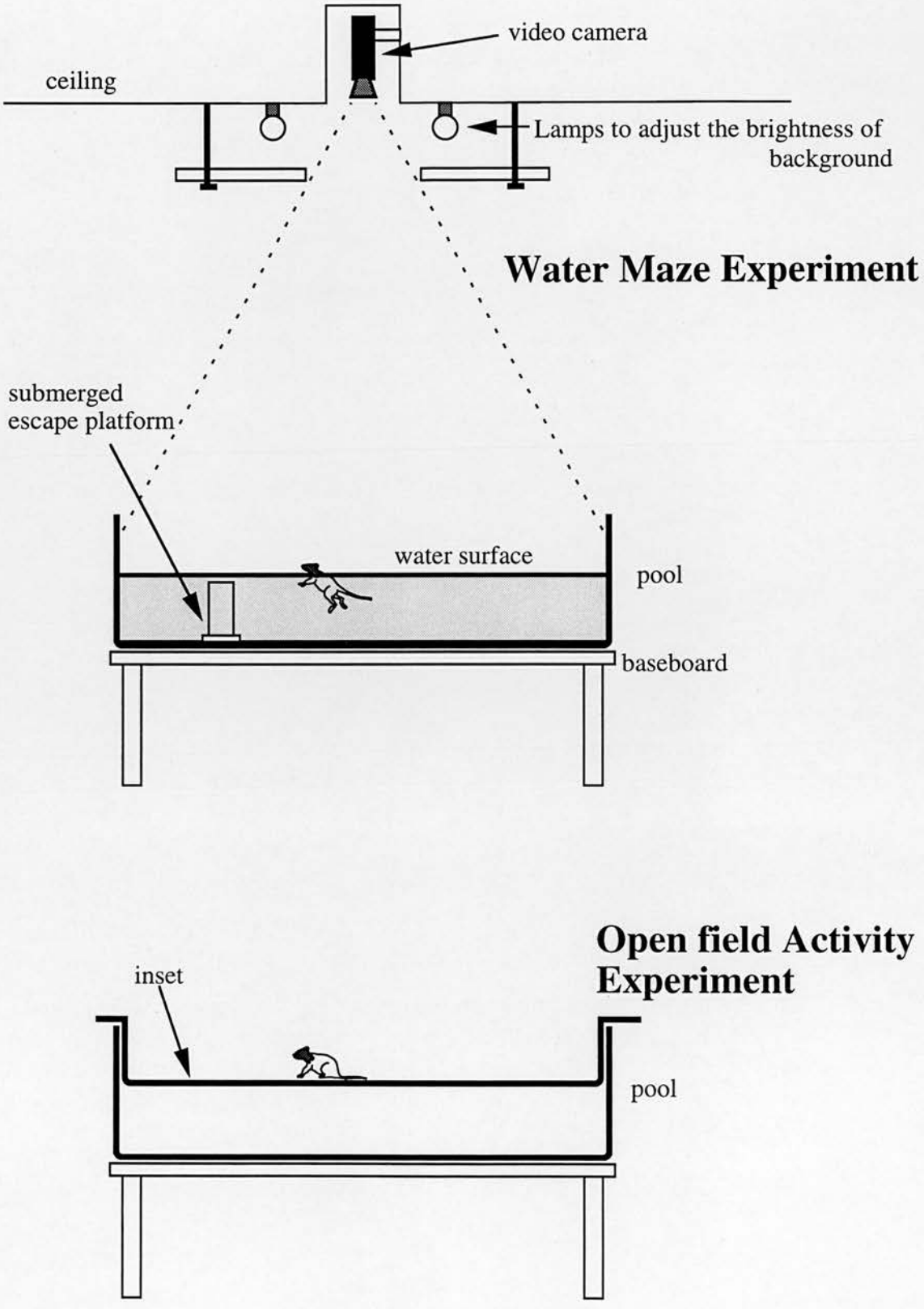


Fig.2-2 Cross section of the system to monitor and record the animal behaviour

2.3.2 Training and testing procedures

The general procedures are shown in Fig. 2-3. The water maze training (place navigation) and testing took 5 days (Day 1 ~ Day 5). In the previous week rats received handling and habituation swimming trials. After the water maze procedure, some rats were used in LTP experiments on Day 6.

(a) Handling

Handling was carried out in the animal house. One or two rats were taken from the home cage and placed on the knees of the experimenter sitting on a stool. The heads of the rats were covered by the experimenter's left hand and the backs of the rats were gently rubbed by the right hand for 3 min. This handling was carried out once a day and repeated 3 times for each rat. The last handling was given on the morning of the day when the habituation trials were carried out.

(b) Habituation

Three days before the commencement of the main place navigation training, the rats grouped in 4 or 5 were divided into individual cages with a paper towel laid on the bottom and received habituation trials. In these trials, the escape platform was removed from the pool. Each rat was placed into the pool and forced to swim for 60 sec without a chance to escape. The water temperature was kept at 25±0.5°C. After the forced swimming, the rat was picked up from the water by hand and returned to the cage with a paper towel, then blown gently with hair dryer to warm their body. This forced swimming was repeated one more time after a 90 sec interval. The rats did not receive any drug treatment in this habituation. After the 2 trials of habituation, each rat was transferred to an individual cage with sawdust and brought back to the animal house. In the following day, every time the swimming training was started,

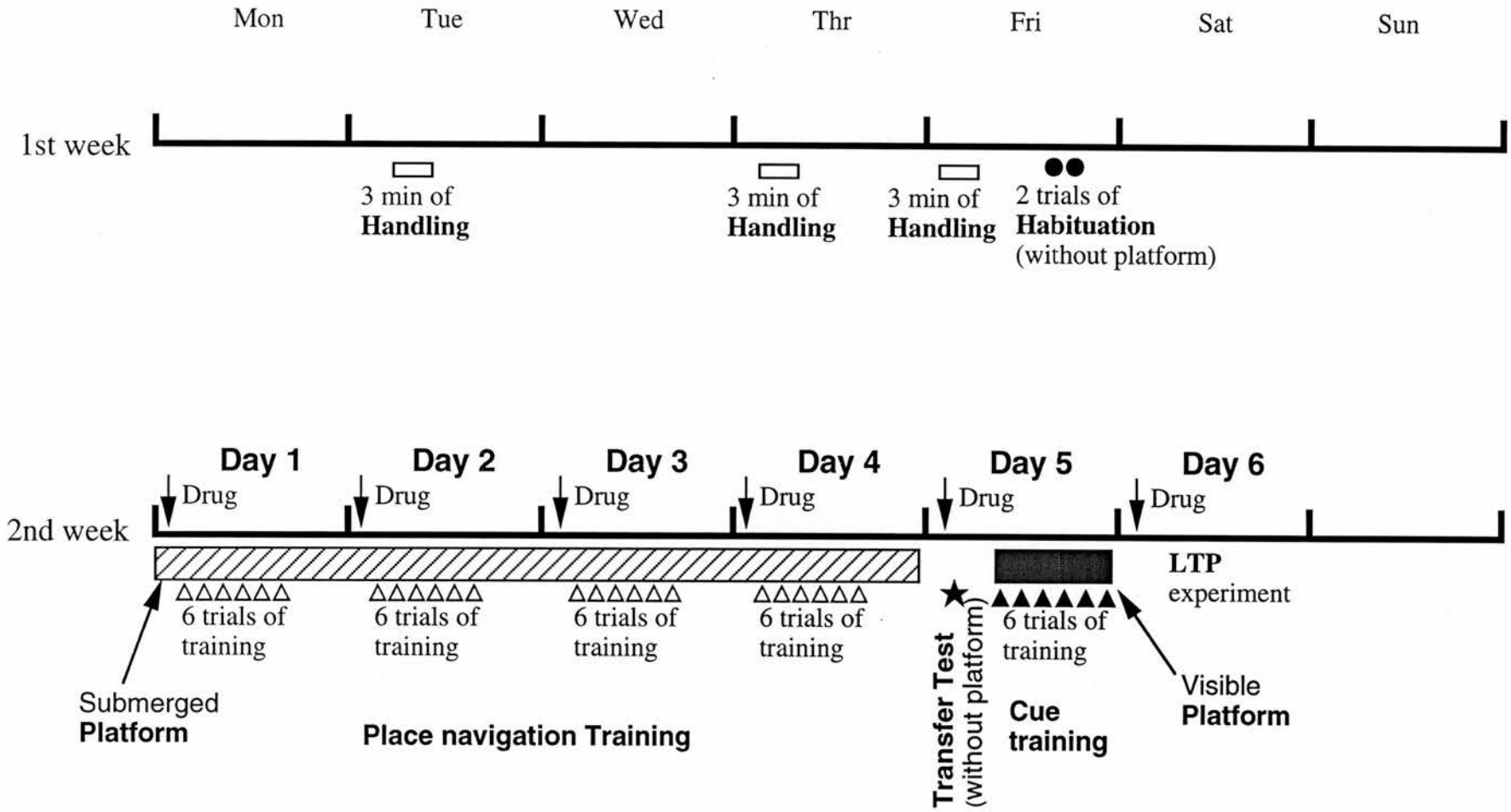


Fig. 2-3 General protocol for Water Maze Experiment

the animals were transferred to the cages with a paper towel.

(c) Hidden platform training (place navigation training)

The place navigation training with hidden escape platform was started 3 days after the habituation trials and took 4 days (Fig.2-3 , Day 1 ~ Day 4). The position of the hidden platform was fixed at one location (NE or SW) throughout the training with half the animals in each group being trained to each position. The animals kept in individual cages were brought out from the animal house to the semi-dark computer operating room next to the pool to have training (Fig.2-1A). Each rat was given 6 trials per day for 4 days (total 24 trials). On each trial, a rat was taken out from the home cage and placed into the water at one of four (N,E,S or W) starting points on the perimeter of the pool. The randomised permutation of the four starting points (N,E,S and W) was designated beforehand. The rat was allowed to swim until it found the submerged escape platform. Once the rat climbed onto the platform, it was left on it for 30 sec and returned to its home cage. If a rat failed to find the platform within 60 sec, the experimenter indicated the position of the platform by hand to assist escape. If a rat showed no intention of escaping to the platform even after the experimenter's instruction, it was picked up from the water and held by hand on the platform for 30 sec. A 60 sec interval (the inter trial interval) was taken at the home cage before the next trial. During the intervals, the rats were occasionally blown with the hair dryer fixed outside of the home cages to maintain their body temperature. The water level was adjusted 1.5 cm above the surface of the hidden platform and the temperature of the pool water was usually kept at 25.0 ± 0.5 °C.

If the animals received drug treatment in this experiment, the drug was dissolved in saline and administered by i.p. 30 or 90 min before the first trial each day.

(d) Transfer test

On Day 5, the platform was removed from the pool. Each rat was placed into the pool at the most distant point (SW or NE) from the location where the platform existed during the training period. The rats were allowed to swim freely for 60 sec to search for the platform. The drug was administered 30 or 90 min before this test.

(e) Visible platform training (cue navigation training)

Immediately after the transfer test, each rat had 6 trials of training to escape to the visible platform. The positions of the platform (NE, SE, SW or NW) and starting points (N, E, S or W) were varied across trials. The randomised combination of starting position and platform position was designated beforehand. Rats were given a 30 sec interval on the platform followed by a 2 min inter-trial interval in their home cages (This inter-trial intervals was 1 min longer than that for hidden platform training because changing the position of the platform after each trial took an extra 1 min.)

(g) LTP experiment

On Day 6, some of the rats which received 10 mg/kg of FR115427 during 5 days of training and testing were submitted to an LTP experiment. The surgical procedure and electrophysiological method of stimulation and recording are described in the following section 2.5. Each rat received a 6th i.p. injection of 10 mg/kg FR, 30 or 90 min before induction of LTP.

2.3.3 Data analysis

(a) Escape latency

At the moment a rat was placed into the water and started to swim, the computer analysis system was started manually by pressing a button set at each starting point of the pool wall. This system was stopped manually by a button on the wall of the room at the moment the rat reached the platform and commenced the motion of climbing onto it, or it stopped automatically if 60 sec had passed before the rat found the platform. The escape latency was defined as the time between the start switch being turned on and the stop switch being turned off.

(b) Escape path length

The escape path length was defined as the length of the track that a rat traversed from the start of swimming and the animal's escape onto the platform. This length was calculated by the sampled x and y coordinate of the rat's position.

(c) Swimming speed

The swimming speed was defined as : Escape path length / escape latency .

(d) Swimming bias during transfer test

The track which a rat traversed during 60 sec transfer test was divided into four parts corresponding to the four quadrants (NW,SW,SE and NE) of the pool (the configuration of each quadrant is shown in Fig.2-1 A). These fragments of the swimming path gave the time spent in each quadrant. The swimming pattern of a rat during the transfer test was expressed as the bias of swimming to each quadrant which is the time spent in each quadrant.

2.3.4 Hippocampal lesion

(a) Surgical procedure

The animal anaesthetised with Tribromoethanol was positioned in a Kopf stereotaxic instrument. Anaesthetic was supplemented as required. After exposing the skull surface by a midline incision, the position of the skull between bregma and lambda was adjusted to be on the same horizontal plane. The skull area overlying the region to be lesioned was dried, A-P and M-L limits are marked, and the bone within this area was removed with a drill.

The stock solution of ibotenic acid was injected into the hippocampus using a 1 μl Hamilton syringe mounted on a stereotaxic frame.

The stereotaxic coordinates for the lesion are given in Table 2-1 which shows 24 separate injection points (12 on each side). The amounts of ibotenic acid solution injected was 0.05 or 0.10 μl. The injection was done by manually at a rate of approximately 0.10 μl/min, and the needle left in place for 1 min following the injection. If the injections were made at the same A-P and M-L location in the different D-V extent, the injection at the most ventral site was made first, the needle was raised after 5-10 sec, and the most dorsal injection was followed 1 min later in order to prevent spread up the tract.

The scalp incision was closed with discontinuous suture and the animal placed in a post operative recovery box overnight. It was then transferred to the individual's normal cage to be given a recovery period of 14 days before commencing training.

(b) Histology

The rats were perfused transcardially with phosphate buffered saline followed by 10% formalin. Horizontal sections of the brain (30 μm thick) were stained with fast cresyl violet to assess cell loss.

Table 2-1

Stereotaxic coordinates for hippocamous lesion

Bregma was used as the zero point for the A-P and M-L coordinates while the D-V measure is taken from the surface of the cortex. Bregma and lambda are on the same horizontal plane. The volume of ibotenic acid injected was 0.05 µl at all sites except for those marked with an * where the amount was 0.10 µl

Coordonates (in mm)

A-P	**M-L**	**D-V**
-2.4	±1.0	-3.4
-3.0	±1.4	-2.6 , -3.4
-3.0	±3.0	-3.0*
-4.0	±2.6	-2.3 , -3.3
-4.0	±3.7	-3.0*
-4.8	±3.9	-7.0*
-5.6	±4.1	-3.8
-5.6	±5.1	-4,0 , -4.9, -5.8

2.4 Open field activity Experiments

2.4.1 Environment and equipment

The system for the water maze experiment was modified for this open field activity experiment. Although the room where this experiment was carried out was not identical to the room used in the water maze experiments, the environment and equipment were similar to that as shown in Fig. 2-1B.

A white painted inset was attached to the empty pool to make a horizontal circular stage at the normal water surface level as shown in Fig.2-2. A rat can walk freely across this circular stage (about 1.9 m in diameter) surrounded by the pool wall (about 30 cm in height). The video camera mounted above the pool was utilized to record the activity of the animal on the stage. As the stage was at the water surface level and painted white, image analyser (HVS Ltd. model VP110) could monitor the position of black head of the rat as in the water maze experiments, and the rat could see similar cues as they saw in the water maze. The track which the animal walked along was recorded and processed by the Archimedes microcomputer system for later analysis.

2.4.2 Experimental procedure

(a) Handling

Handling was carried out before the activity test. The procedure of the handling was the same as in water maze experiments. One or two rats were held on the knees of the experimenter and their backs were rubbed gently for 3 min. This handling was

carried out once a day and repeated 3 times for each rat. The last handling was given on the morning of the day of the activity recording.

(b) Habituation and recording of activity

The experiment was run in replicates, consisting of habituation and activity recording where 4 animals were used per replicate. The 4 animals were housed in the same cage. Each animal had 30 min habituation and 10 trials of activity recording along the time schedule shown in Fig. 2-4.

In habituation, all 4 animals were placed on the stage and allowed to explore the testing environment for 30 min before being taken back to their home cage.

About 5 min later, the first animal was placed on the stage and left for 2 min to record its behaviour in the "pre-drug state" on a videotape. Immediately after this recording, the first rat received an i.p. injection of drug and was returned to its home cage. Subsequently, the second rat was placed on the stage to have 2 min activity recording followed by a drug treatment. The third and fourth rats also had the activity recording and drug treatment. This activity recording and drug treatment of all 4 animals was completed within 10 min. Exactly 10 min after the drug treatment, the first rat was brought back to the stage and again left for 2 min to record its behaviour. This behaviour was analysed as "10 min post-drug state". The behaviour of the second, third and fourth rat was also recorded 10 min after the drug treatment respectively. The same procedure was repeated at 10 min intervals until the completion of the recording of "90 min post-drug state" of all 4 animals.

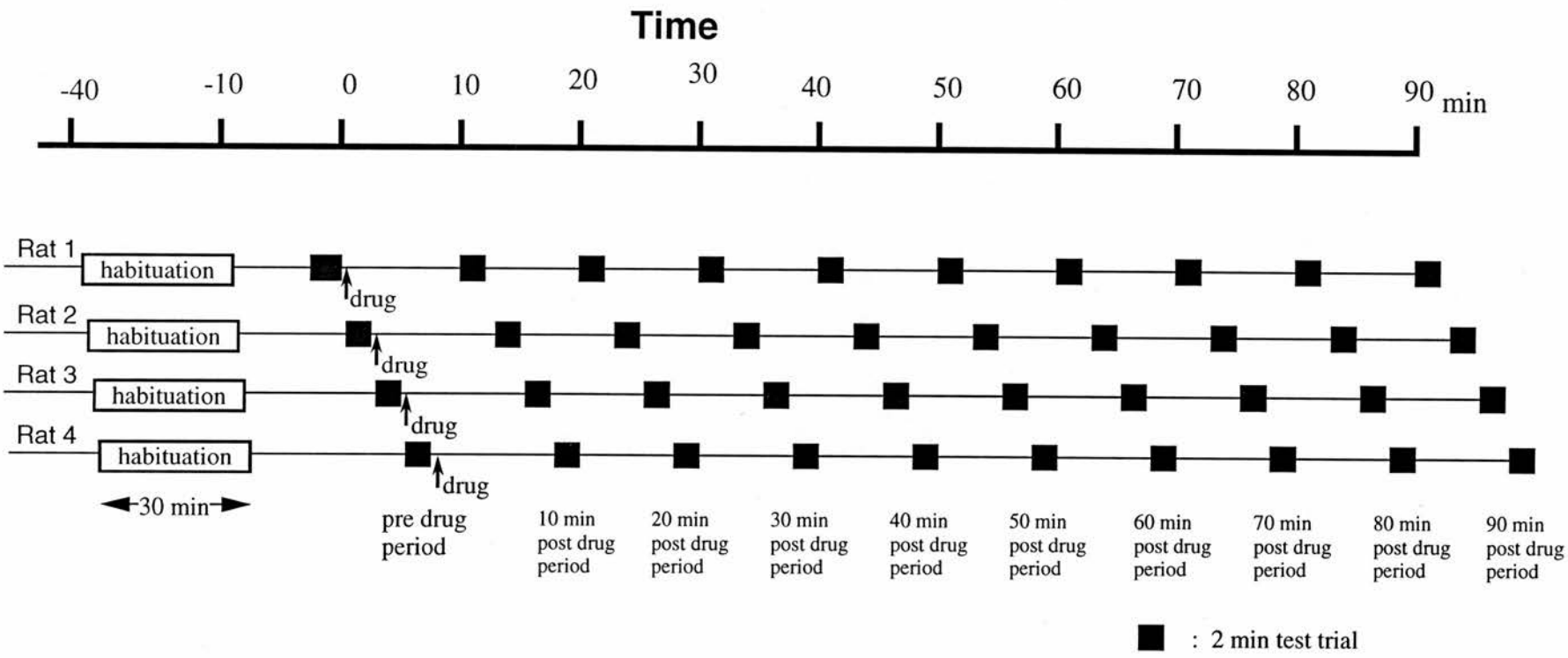


Fig. 2-4 Time schedule for Openfield Activity Experiment

2.4.3 Analysis of behaviour

As measures of activity, 4 indices (ataxia, head weaving, body rolling and locomotor activity) were adopted. Ataxia, head weaving and body rolling were scored by observing the video monitor replaying each 2 min record of animal activity. The scorer was blind to the drug treatment given to each animal. The reliability of the scoring system was checked by comparison between the scoring of the experimenter and an experienced specialist, Dr Allan Young in the Department of Pharmacology, University of Edinburgh. The reproducibility of scoring was checked by repeated scoring of the same videotape.

Locomotor activity was quantified by computer analysis of the track of animal movements.

(a) Ataxia

Ataxia was rated on a 4 point scale from 0 to 3. A rating of 0 was given if ataxia was absent, i.e. the animal showed normal walking activity. A rating of 1 was given if ataxia was equivocal, i.e. the animal showed a slight awkwardness of movement such as tottering or stumbling. A rating of 2 was given if ataxia was present, i.e. the animal showed an apparent impairment of righting reflexes indicated by partial abduction of hindlimbs resulting in frequent falling on one side but the animal could still move forward. A rating of 3 was given if ataxia was intense, i.e. the animal showed almost complete cessation of locomotion with abduction of fore- and hindlimbs. The animal may change the direction of its body or occasionally roll onto its side or raised its head.

(b) Head weaving

Head weaving was scored by counting the total number of lateral head movements during the 2 min recording period. Each count was given if the head of the animal was shifted more than 45° from the midline of the body.

(c) Body rolling

This index was scored by counting the total number of side-to-side movements of the hind quarters with the detachment of the sole of 4 limbs from the stage floor during the 2 min recording period.

(d) Locomotor activity

Quantification of locomotor activity was done by calculating the total length of the trace that the animals traversed during each 2 min recording period. Thus, locomotor activity was expressed as meters per 2 min.

2.5 LTP Experiments

2.5.1 Surgery

Rats were anaesthetised with Urethane (1.5 g/kg i.p.) and placed in a Kopf stereotaxic device. A midline incision along the scalp was made to expose the skull surface. Holes were drilled in the skull for electrodes and a stainless steel screw which was placed anterior to bregma and served as the earth.

A stimulating electrode, consisting of two Teflon-coated stainless steel wires (110 μm diameter) twisted together and separated horizontally at the tip by approximately 1.0 mm, was implanted in the perforant path. A recording electrode, consisting of a single Teflon-coated stainless steel wire (110 μm diameter) was implanted in the hilus of the dentate gyrus. The coordinates, relative to bregma were 3.5 mm posterior and 2.0 mm lateral for the recording electrode and 8.0 mm posterior and 4.0 mm lateral for the stimulating electrode. The electrodes were lowered to optimise the amplitude of evoked field responses of granule cells (the depth of stimulating electrode and recording electrode were approximately 2.7 mm and 1 mm respectively). The electrode placement and recording were sometimes made bilaterally.

2.5.2 Electrophysiological procedure

A programmable stimulator (Neurolog NL-800, Digitimer) delivered constant 0.7 mA current monopolar stimuli (100 μsec biphasic, square wave pulses) at 20 sec intervals throughout the experiment. The evoked field excitatory post synaptic potential (EPSP) was amplified and filtered between 1 Hz and 10 kHz using a Grass EEG amplifier. The signals were converted from analog to digital in a Minc-11/23 computer and the data stored on magnetic disks. The slope of the early-rising part of the evoked field-potential was measured using linear regression (Fig. 4-1 page97).

After a stable response was observed for more than 15 min, a pre-drug baseline response was recorded for 10 min and the drug was then administered i.p. The drugs (MK-801 and FR115427) were made up in 0.9 % saline. A high frequency tetanus, consisting of 5 trains of 400 Hz 50 msec stimulation spaced 10 sec apart, was given after a 30 min or 90 min interval. The slope of the EPSP was analysed as the sole

index of synaptic efficacy in the dentate gyrus. The body temperature of the anaesthetised animals was kept within ± 0.1°C from the initial body temperature (36.1 ~ 36.8°C) using an electric blanket and a desk lamp throughout the recording period.

2.6 Data analysis

In the analysis of parametric data, ANOVA followed by Dunnett's multiple comparison test was carried out to compare one control group and other drug groups.

Tukey's honestly significant difference test was carried out to compare two arbitrary selected groups.

The results of the ANOVA is described in the following formula.

[F(a, b)=c p value]

a: degree of freedom in the experimental condition

b: degree of freedom in the residual error

c: F value

= mean of square of condition's effect / mean of square of residual error

p value is a probability of type 1 error (rejecting null hypothesis when it is true)

In the analysis of non-parametric data, Kruskal-Wallis ANOVA followed by Wilcoxon test was carried out.

Fisher's exact test was applied to the analysis of frequency of a particular event in the experiments.

Chapter 3
Experiment 1: Open Field Activity Test

3.1 Introduction

The aim of this experiment was to examine whether systemic administration of FR115427 produced MK-801-like motor effects in rats. The Open Field Activity Test was designed to analyse behavioural changes by four indices, ataxia, locomotor activity, head weaving and body rolling which had been described in other investigations evaluating characteristic changes induced by NMDA receptor antagonists (Tricklebank et al., 1988; Hiramatsu et al., 1989; Löscher and Hönack, 1992). As a wider range of drug doses were testable as well as the time course of the drug's effect, compared to water maze or LTP experiments, this open field activity test was carried out first. The objective was to find a suitable drug dose and timing of administration for subsequent experiments.

In order not to give excessive stress to the animals in the behavioural test compared with the animals in the water maze task, handling and habituation trial was given to the animals as in the water maze experiments and the tests were carried out on the specially designed stage set in the water pool under the same lighting condition as in the maze training.

3.2 Brief Procedure of Experiment

Details of the method were described in Chapter 2. In this section, the outline of procedure is summarised.

In advance to the test trial, Lister hooded rats received handling and habituation sessions in which animals were allowed to explore the experimental environment (white circular stage set in the water maze pool) for 30 min (see Fig. 2-2). The stage was illuminated by soft diffuse lights. Following the habituation, the animals received repetitive 2 min behavioural test trials. In each test trial, the rat was placed on the stage and its behaviour recorded on the video tape by a camera mounted in the ceiling above the stage. At the end of the 2 min test period, the animal was brought back to its home cage. The animals were allocated to one of the 9 groups (saline, MK 1 mg/kg, MK 0.32 mg/kg, MK 0.1 mg/kg, MK 0.032 mg/kg, FR 32 mg/kg, FR 10 mg/kg, FR 3.2 mg/kg, and FR 1 mg/kg). Each group consisted of 8 animals. Each animal received i.p. injections of drug only once. The behavioural test trial was carried out just before the drug injection and repeated at 10 min intervals after drug injection until 90 min post drug period (see Fig. 2-4).

The recorded behaviour was analysed and quantified by the following indices, ataxia, head weaving body rolling and locomotor activity. The score of ataxia, head weaving and body rolling were rated manually by observing video monitor. Ataxia was rated on a four point scale of 0 to 3. The score for head weaving is a direct count of lateral head movement in 2 min. The score for body-rolling is a direct count of side-to-side movements of the hind quarters in 2 min. Locomotor activity was automatically measured by the image analyzing system which expresses the activity by the distance that the animals traversed across the stage in 2 min period.

The scorer was blind to the drug treatment and the validity of scoring was checked by Dr Allan Young in the Department of Pharmacology.

3.3 Results

High doses of MK-801 (1 ~ 0.32 mg/kg) and FR115427 (32 ~ 10 mg/kg) induced marked ataxia, head weaving immediately after i.p. administration. Locomotor activity of rats receiving the highest dose of MK-801 or FR115427 was initially inhibited by severe ataxia then gradually enhanced with the recovery from the ataxic effect. Those rats showed body rolling as their locomotor activity recovered.

As summarised in Table 3-1, overall one-way between- and within-subjects ANOVAs of each behavioural score suggested that MK-801 and FR 115427 induced significant changes in the scores for ataxia, head weaving, body rolling and locomotor activity (a significant group effect at the level of $p < 0.0001$). These changes were also suggested to be time dependent (the effect of *trial was significant at $p < 0.0001$) (*: see Fig. 2-4). The significance ($p < 0.0001$) in interaction terms (group x trial) suggests the adequacy of independent analysis at each trial. Kruskal-Wallis Nonparametric one-way ANOVA followed by Wilcoxon test was applied to analyse the scores for ataxia, head weaving and body rolling. A one-way between-subjects parametric ANOVA followed by Dunnett's multiple comparison test was carried out to compare the locomotor activity of the saline group and that of drug groups, and a one-way within-subjects ANOVA followed by Dunnett's multiple comparison test was performed to analyse the time dependent change in locomotor activity.

As described below, all scores during the pre-drug period indicated no significant difference between groups.

Table 3-1

Over-all one-way between- and one-way within-subjects ANOVA

Ataxia

source of variation	df	sum of squares	mean square	F	p
Group	8	468.625	58.578	108.782	0.0000
Error	63	33.925	0.538		
Trial	9	33.078	3.675	54.022	0.0000
Group × Trial	72	85.345	1.185	17.423	0.0000
Error	567	38.575	0.068		

Head Weaving

source of variation	df	sum of squares	mean square	F	p
Group	8	26024.194	3253.024	20.402	0.0000
Error	63	1045.000	159.444		
Trial	9	4204.644	467.183	8.067	0.0000
Group × Trial	72	18825.556	261.466	4.515	0.0000
Error	567	32837.000	57.914		

Body Rolling

source of variation	df	sum of squares	mean square	F	p
Group	8	1218.519	152.315	15.552	0.0000
Error	63	617.013	9.794		
Trial	9	104.446	11.605	3.380	0.0000
Group × Trial	72	905.842	12.581	3.665	0.0000
Error	567	1946.613	3.433		

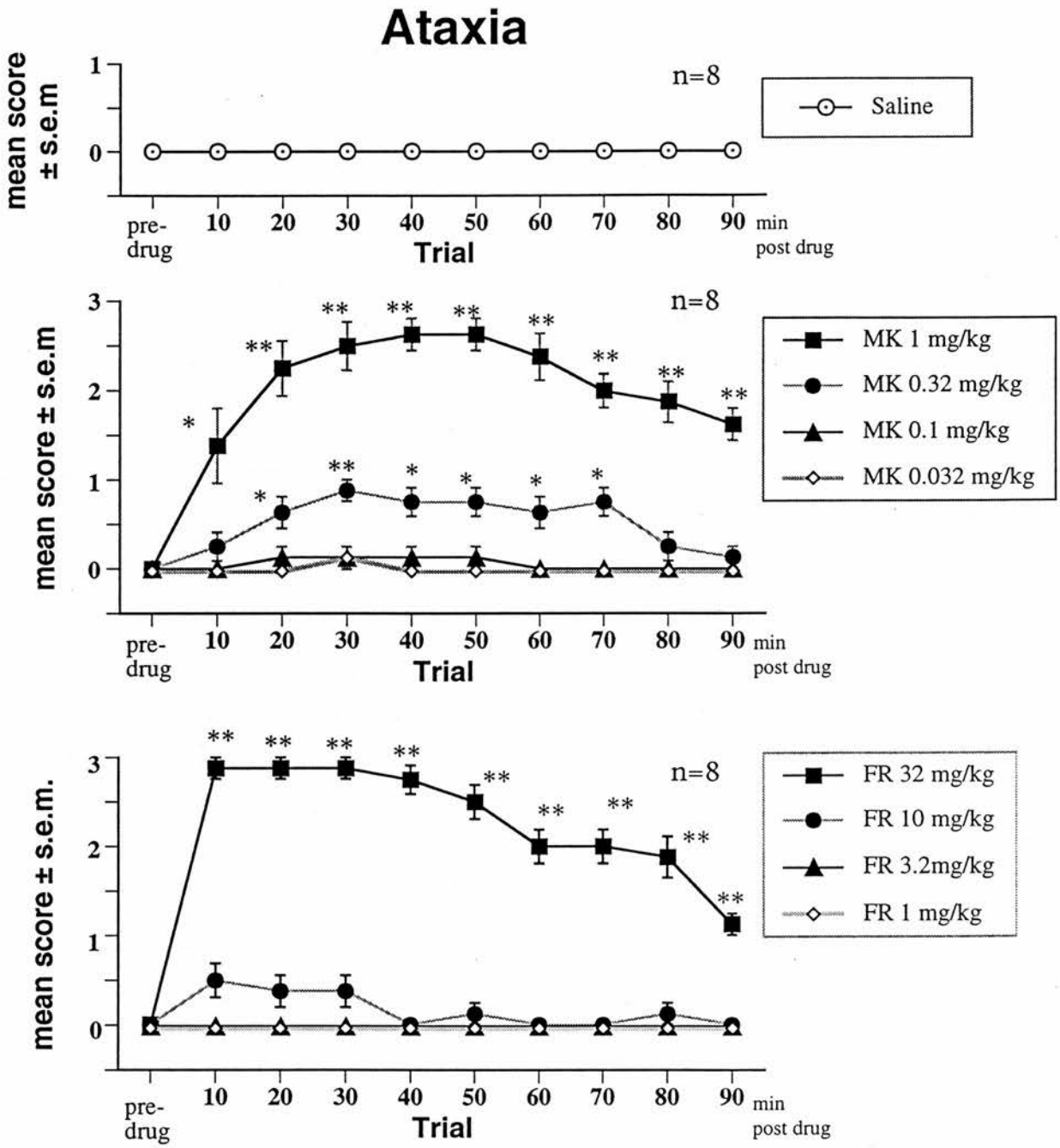
Locomotor activity

source of variation	df	sum of squares	mean square	F	p
Group	8	7940.400	992.550	7.918	0.0000
Error	63	7897.115	125.351		
Trial	9	846.932	94.104	5.550	0.0000
Group × Trial	72	5857.126	81.349	4.789	0.0000
Error	567	9613.104	16.954		

3.3.1 Ataxia

The time course of the mean (± standard error of the mean: s.e.m.) ataxic sore of each group is shown in Fig. 3-1. An overall one-way between- and within-subjects ANOVA of ataxic scores revealed significant effects of group ($p < 0.0001$), trial ($p < 0.0001$) and group x trial interaction ($p < 0.0001$) (Table 3-1). All animals were scored 0 (= no sign of ataxia) at the pre-drug trial. Kruskal-Wallis ANOVA performed at each post-drug trial shows significant group effects ($p < 0.0001$) at all trials (between 10 min and 90 min post drug period) (Table 3-2). The scores of the saline group were 0 throughout the experiment. The i.p. injection of 1 mg/kg of MK-801 induced severe ataxia (Fig. 3-1). Comparison by Wilcoxon test between the MK-801 1 mg/kg group and the saline group indicated a significant effect at all post drug periods ($p < 0.05$ at 10 min. $p< 0.01$ between 20 and 90 min.) (Table 3-2). The score of the 0.32 mg/kg group may not be the same as the saline group at the level of $p < 0.01$ between the 20 min and 70 min post injection (at 30 min, $p < 0.001$) using a Wilcoxon test. The repeated same tests showed that 0.1 or 0.032 mg/kg MK-801 did not induce any significant ataxic effect at any period after injection.

I.p. administration of 32 mg/kg FR115427 induced rapid and strong ataxia. Within 10 min, the ataxic index reached an almost maximal score (Fig. 3-1), with 7 out of 8 animals in the condition of rate 3 ataxia during the first 30 min. A recovery from the severe ataxia of this group was faster than that of the 1 mg/kg MK-801 group. At 90 min, 7 out of 8 animals only rated a score of 1. The difference in the ataxic score between this group and control group was found to be highly significant ($p < 0.001$) in the multiply performed Wilcoxon test (Table 3-2) throughout the 90 min post injection periods. 10 mg/kg of FR115427 induced a weak ataxia (mean score < 1) (Fig.3-1). Wilcoxon test suggested that the score of this group was not significantly greater than 0 (= score of saline group) at 10, 20, 30 and 50 min after



** : $p < 0.01$ compared to saline group (Wilcoxon Test)

* : $p < 0.05$ compared to saline group (Wilcoxon Test)

Fig. 3-1 Comparison of ataxia induced by saline MK-801 and FR115427 in rats. The rating scale was described in Materials and Methods in chapter 2. Values are the means ± s.e.m. of eight animals

Table 3-2
Kruskal-Wallis Nonparametric ANOVA of Ataxic scores and
One sample nonparametric test (Wilcoxon Test)

Ataxia

Trial (min)	10	20	30	40	50	60	70	80	90
Kruskal-Wallis (KW) statistics	51.559	57.578	59.182	64.024	61.875	61.839	65.607	61.571	66.222
P value (KW) (p <)	.0001***	.0001***	.0001***	.0001***	.0001***	.0001***	.0001***	.0001***	.0001***
saline median	0	0	0	0	0	0	0	0	0
MK 1.0 mg/kg median	1	2.5	3	3	3	2.5	2	2	2
Sum of positive ranks	21	36	36	36	36	36	36	36	36
No. of pairs	6	8	8	8	8	8	8	8	8
One tailed P value	.0156 *	.0039**	.0039**	.0039**	.0039**	.0039**	.0039**	.0039**	.0039**
MK 0.32 mg/kg median	0	1	1	1	1	1	1	0	0
Sum of positive ranks	3	15	28	21	21	15	21	3	1
No. of pairs	2	5	7	6	6	5	6	2	1
One tailed P value	.25	.0312 *	.0078**	.0156 *	.0156 *	.0312 *	.0156 *	.25	.50
MK 0.1 mg/kg median	0	1	1	1	1	1	1	0	0
Sum of positive ranks	-	1	1	1	1	1	-	-	-
No. of pairs	-	1	1	1	1	1	-	-	-
One tailed P value	-	.50	.50	.50	.50	.50	-	-	-
MK0.032mg/kg median	0	0	0	0	0	0	0	0	0
Sum of positive ranks	-	-	1	-	-	-	-	-	-
No. of pairs	-	-	1	-	-	-	-	-	-
One tailed P value	-	-	.50	-	-	-	-	-	-
FR 32 mg/kg median	3	3	3	3	2.5	2	2	2	1
Sum of positive ranks	36	36	36	36	36	36	36	36	36
No. of pairs	8	8	8	8	8	8	8	8	8
One tailed P value	.0039**	.0039**	.0039**	.0039**	.0039**	.0039**	.0039**	.0039**	.0039**
FR 10 mg/kg median	0.5	0	0	0	0	0	0	0	0
Sum of positive ranks	10	6	6	-	1	-	-	-	-
No. of pairs	4	3	3	-	1	-	-	-	-
One tailed P value	.0625	.125	.125	-	.50	-	-	-	-
FR 3.2 mg/kg median	0	0	0	0	0	0	0	0	0
Sum of positive ranks	-	-	-	-	-	-	-	-	-
No. of pairs	-	-	-	-	-	-	-	-	-
One tailed P value	-	-	-	-	-	-	-	-	-
FR 1.0 mg/kg median	0	0	0	0	0	0	0	0	0
Sum of positive ranks	-	-	-	-	-	-	-	-	-
No. of pairs	-	-	-	-	-	-	-	-	-
One tailed P value	-	-	-	-	-	-	-	-	-

-: not possible to analyse because SD value is 0
No. of pairs: 0 values were excluded from calculations

injection ($p > 0.05$). The animals which received 3.2 or 1 mg/kg FR115427 did not show any sign of ataxia during the 90 min.

3.3.2 Head weaving

As shown in Fig.3-2, the saline group showed a stable and low level of head-weave counts throughout the experimental period. An overall one-way between- and within-subjects ANOVA of head weaving scores revealed significant effects of group ($p < 0.0001$), trial ($p < 0.0001$) and group x trial interaction ($p < 0.0001$) (Table 3-1). Kruskal-Wallis nonparametric ANOVA revealed significant group effect at 10, 20, 30, 40, 50, 60 and 80 min post drug period (Table 3-3). At these periods, Wilcoxon test was performed to compare the score of each group with the saline group (Table 3-3).

In the MK-801 (1 mg/kg) group, the count of head weaving significantly increased at 10 min ($p < 0.01$), 30, 40, 50 min ($p < 0.05$) and 60, 80 min ($p < 0.01$) (Table 3-3, Fig. 3-2). The time course of the above score was complicated because the ataxia reduced the head-weave score by inhibiting the lateral movement of the animal's head. The count was especially suppressed at 20 and 30 min. During this period, the animals still moved their head very rapidly but the lateral shift of the head was restricted and too small to satisfy the counting criteria (the shift larger than 45°). The 0.32 mg/kg dose of MK-801 significantly ($p < 0.05$) increased the count of head weaving at all periods after injection except 70 and 90 min (Table 3-3, Fig 3-2), while neither 0.1 nor 0.032 mg/kg of MK-801 increased the frequency of head weaving significantly.

The 32 mg/kg dose of FR induced significant increase in head weaving between 30 min and 80 min except at 70 min after injection ($p < 0.05 \sim 0.01$

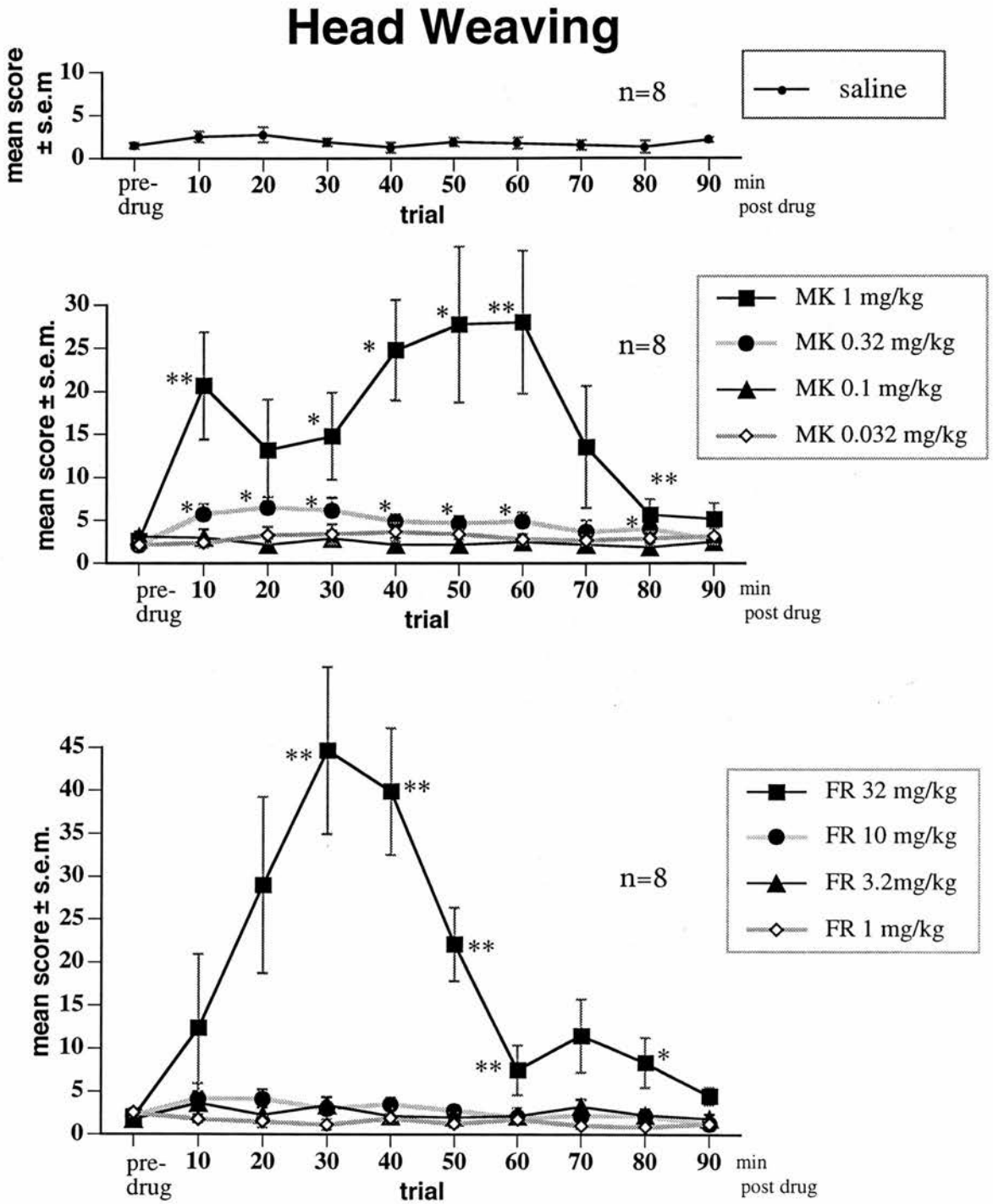


Fig. 3-2 Comparison of head weaving induced by saline MK-801 and FR115427 in rats. The rating scale was described in Materials and Methods in chapter 2. Values are the means ± s.e.m. of eight animals

Table 3-3

Kruskal-Wallis Nonparametric ANOVA of Head Weaving scores and One sample nonparametric test (Wilcoxon Test)

Head Weaving

Trial (min)	pre-	10	20	30	40	50	60	70	80	90
Kruskal-Wallis (KW) statistics	7.630	23.217	15.982	31.397	37.132	35.132	32.136	15.387	17.350	10.999
P value (KW)	.470	.003**	.0426*	.0001***	.0001***	.0001***	.0001***	.052	.0267*	.202
saline median	1.5	2.5	2.5	2.0	1.0	2.0	1.5	1.0	0.5	2.0
MK 1.0 mg/kg median	2.0	12.0	6.5	12.5	23	26.5	19.5	5.5	4.5	2.5
Sum of positive ranks	30.0	36	27.5	32.5	35	34.5	36	26.5	36	18
Sum of negative ranks	-6.0	0	-8.5	-3.5	-1	-1.5	0	-1.5	0	-10
No. of pairs	8	8	8	8	8	8	8	7	8	7
Two tailed P value	0.109	.0078**	0.1953	.0391*	.0156*	.0156*	.0078**	0.031	.0078**	.578
MK 0.32 mg/kg median	2.0	4.5	8.0	5.0	5.5	5.0	5.5	3.0	4.0	2.5
Sum of positive ranks	25.5	34.5	34.0	21.0	28.0	33.5	34.0	26.5	34.5	1.0
Sum of negative ranks	-10.5	-1.5	-2.0	0.0	0.0	-2.5	-2.0	-1.5	-1.5	17.5
No. of pairs	8	8	8	6	7	8	8	7	8	-10.5
Two tailed P value	0.313	.0156*	.0234*	.0312*	.0156*	.0234*	.0234*	0.031	.0156*	0.578
MK 0.1 mg/kg median	3.0	3.0	2.5	2.0	2.0	2.0	2.5	1.5	1.0	2.0
Sum of positive ranks	32.5	20.5	15.5	23.0	22.0	11.5	27.0	19.0	33.0	10.5
Sum of negative ranks	-3.5	-15.5	-20.5	-13.0	-6.0	-9.5	-9.0	-9.0	-3.0	-4.5
No. of pairs	8	8	8	8	7	6	8	7	8	5
Two tailed P value	0.039	.742	.742	.547	.219	.844	.250	.469	.0391 *	.438
MK0.032mg/kg median	1.5	2.0	3.0	3.0	3.0	4.0	3.0	2.0	3.0	2.5
Sum of positive ranks	24.0	16.5	23.0	20.0	20.0	24.5	30.0	15.0	33.0	16.0
Sum of negative ranks	-12.0	-19.5	-13.0	-8.0	-1.0	-3.5	-6.0	0.0	-3.0	-5.0
No. of pairs	8	8	8	7	6	7	8	5	8	6
Two tailed P value	0.461	.844	.547	.375	.063	.078	.109	.063	.0391*	.313
FR 32 mg/kg median	2.0	0.0	23.0	41.0	34.0	22.0	3.5	14.5	6.5	4.0
Sum of positive ranks	29.0	15.0	31.0	36.0	36.0	36.0	34.0	15.0	33.0	25.0
Sum of negative ranks	-7.0	-21.0	-5.0	0.0	0.0	0.0	-2.0	0.0	-3.0	-3.0
No. of pairs	8	8	8	8	8	8	8	5	8	7
Two tailed P value	.148	.742	.078	.0078**	.0078**	.0078**	.0234*	.063	.0391*	.078
FR 10 mg/kg median	2.5	2.0	3.5	2.5	4.0	2.5	1.5	1.5	2.5	3.0
Sum of positive ranks	26.5	18.5	27.5	19.5	27.0	16.0	24.0	17.0	34.0	2.5
Sum of negative ranks	-9.5	-17.5	-8.5	-8.5	-1.0	-5.0	-12.0	-4.0	-2.0	-18.5
No. of pairs	8	8	8	7	7	6	8	6	8	6
Two tailed P value	.250	.945	.195	.375	.0312*	.313	.461	.219	.0234*	.094
FR 3.2 mg/kg median	1.0	3.0	1.5	3.5	1.0	1.5	1.5	3.5	2.0	2.0
Sum of positive ranks	20.0	21.0	14.0	21.0	12.0	10.5	21.0	20.0	32.0	9.0
Sum of negative ranks	-16.0	-15.0	-22.0	-7.0	-3.0	-10.5	-15.0	-1.0	-4.0	-12.0
No. of pairs	8	8	8	7	5	6	8	6	8	6
Two tailed P value	.844	.742	.641	.297	.313	1.000	.742	.063	.055	.844
FR 1.0 mg/kg median	3.0	1.5	1.0	0.0	2.0	1.0	1.0	1.0	1.0	1.5
Sum of positive ranks	30.0	6.0	9.0	6.0	23.0	2.5	13.0	3.0	28.0	1.5
Sum of negative ranks	-6.0	-30.0	-27.0	-15.0	-5.0	-18.5	-23.0	-3.0	-8.0	-13.5
No. of pairs	8	8	8	6	7	6	8	3	8	5
Two tailed P value	.109	.109	.250	.438	.156	.094	.547	1.000	.195	.125

No. of pairs: 0 values were excluded from calculations

Kruskal-Wallis ANOVA indicated that group effect is not significant

Table 3-3). At 10 and 20 min period the animals moved their head rapidly but the ataxic effect prevented the lateral shift of the head from exceeding the counting criteria. Although the Wilcoxon test detected a significant increase of score in the 10 mg/kg FR group at 40 and 80 min when the score of the saline group was unusually low, 10 mg/kg or lower doses of FR did not significantly increase the count of head weaving (Fig. 3-2, Table 3-3).

3.3.3 Body rolling

No body rolling was observed in the saline group throughout the experiment. Before drug administration, no animals in any group showed any sign of body rolling. An overall one-way between- and within-subjects ANOVA of body rolling scores revealed significant effects of group ($p < 0.0001$), trial ($p < 0.0001$) and group x trial interaction ($p < 0.0001$) (Table 3-1).

As shown in the Fig. 3-3, the significant group effect is due to an increase of body rolling scores in MK 1 mg/kg and FR 32 mg/kg groups. Wilcoxon tests following Kruskal-Wallis nonparametric ANOVAs confirmed a significant effect in MK 1 mg/kg and FR 32 mg/kg groups (Table 3-4). In these groups, the increase of body rolling scores were suppressed by ataxic effects during the early phase following drug administration (Fig. 3-3). The severe ataxia abolished the chance of body rolling as well as the locomotor activity of animals. As the animals recovered from ataxia, their locomotor activity and body rolling scores increased (compare Fig. 3-1, 3-3 and 3-4).

At the second highest dose of these drugs, 0.32 mg/kg of MK-801 and 10 mg of FR 115427, a slight increase in the score was observed but the change was not statistically significant (Table 3-4, Fig. 3-3). At low doses (0.1, 0.032 mg/kg MK

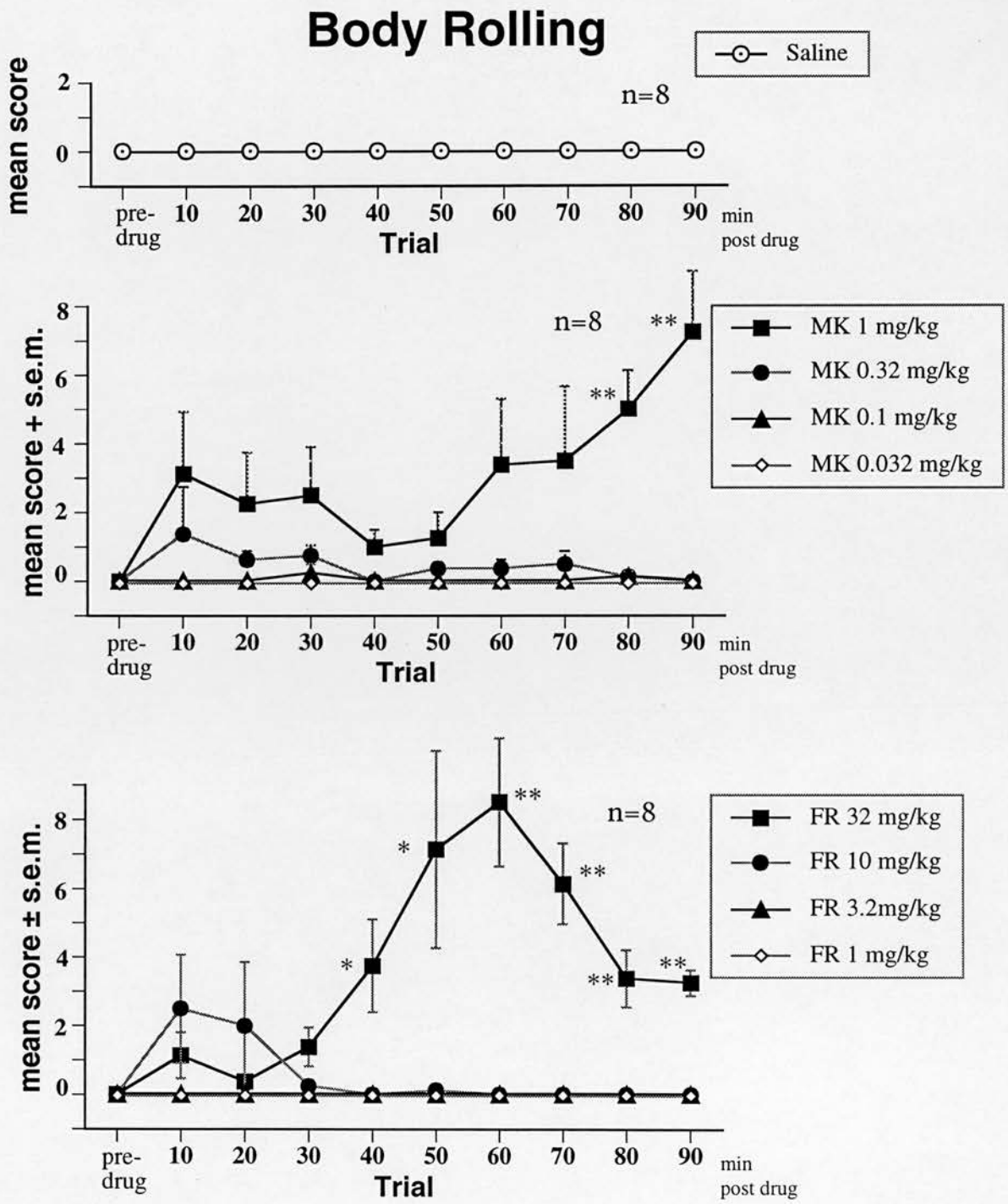


Fig. 3-3 Comparison of body rolling induced by saline MK-801 and FR115427 in rats. The rating scale was described in Materials and Methods in chapter 2. Values are the means ± s.e.m. of eight animals

Table 3-4

Kruskal-Wallis Nonparametric ANOVA of Body Rolling scores and One sample nonparametric test (Wilcoxon Test)

Body Rolling

Trial (min)	10	20	30	40	50	60	70	80	90
Kruskal-Wallis (KW) statistics	22.545	19.071	19.330	34.148	26.882	39.170	65.607	58.794	64.256
P value (KW) (p <)	.01**	.05*	.05***	.0001***	.001***	.0001***	.0001***	.0001***	.0001***
saline median	0	0	0	0	0	0	0	0	0
MK 1.0 mg/kg median	1	0	0	0	0	1	1	4.5	7
Sum of positive ranks	10	6	6	6	6	10	10	36	28
No. of pairs	4	3	3	3	3	4	4	8	7
One tailed P value	.0625	.125	.125	.125	.125	.0625	.0625	.0039**	.0078**
MK 0.32 mg/kg median	0	0.5	0.5	0	0	0	0	0	0
Sum of positive ranks	1	10	10	-	6	3	3	1	-
No. of pairs	1	4	4	-	3	2	2	1	-
One tailed P value	.50	.0625	.0625	-	.125	.25	.25	.50	-
MK 0.1 mg/kg median	0	0	0	0	0	0	0	0	0
Sum of positive ranks	-	-	1	-	-	-	-	1	-
No. of pairs	-	-	1	-	-	-	-	1	-
One tailed P value	-	-	.50	-	-	-	-	.50	-
MK0.032mg/kg median	0	0	0	0	0	0	0	0	0
Sum of positive ranks	-	-	-	-	-	-	-	-	-
No. of pairs	-	-	-	-	-	-	-	-	-
One tailed P value	-	-	-	-	-	-	-	-	-
FR 32 mg/kg median	0	0	1	3	6	8	6	3.5	3
Sum of positive ranks	6	6	10	15	15	28	36	28	36
No. of pairs	3	3	4	5	5	7	8	7	8
One tailed P value	.125	.125	.0625	.0312 *	.0312 *	.0078**	.0039**	.0078**	.0039**
FR 10 mg/kg median	0.5	0	0	0	0	0	0	0	0
Sum of positive ranks	10	3	3	-	1	-	-	-	-
No. of pairs	4	2	2	-	1	-	-	-	-
One tailed P value	.0625	.25	.25	-	.50	-	-	-	-
FR 3.2 mg/kg median	0	0	0	0	0	0	0	0	0
Sum of positive ranks	-	-	-	-	-	-	-	-	-
No. of pairs	-	-	-	-	-	-	-	-	-
One tailed P value	-	-	-	-	-	-	-	-	-
FR 1.0 mg/kg median	0	0	0	0	0	0	0	0	0
Sum of positive ranks	-	-	-	-	-	-	-	-	-
No. of pairs	-	-	-	-	-	-	-	-	-
One tailed P value	-	-	-	-	-	-	-	-	-

-: not possible to analyse because SD value is 0

No. of pairs: 0 values were excluded from calculations

and 3.2, 1 mg/kg FR), animals were scored 0 except one animals in the 0.1 mg/kg MK group.

3.3.4 Locomotor activity

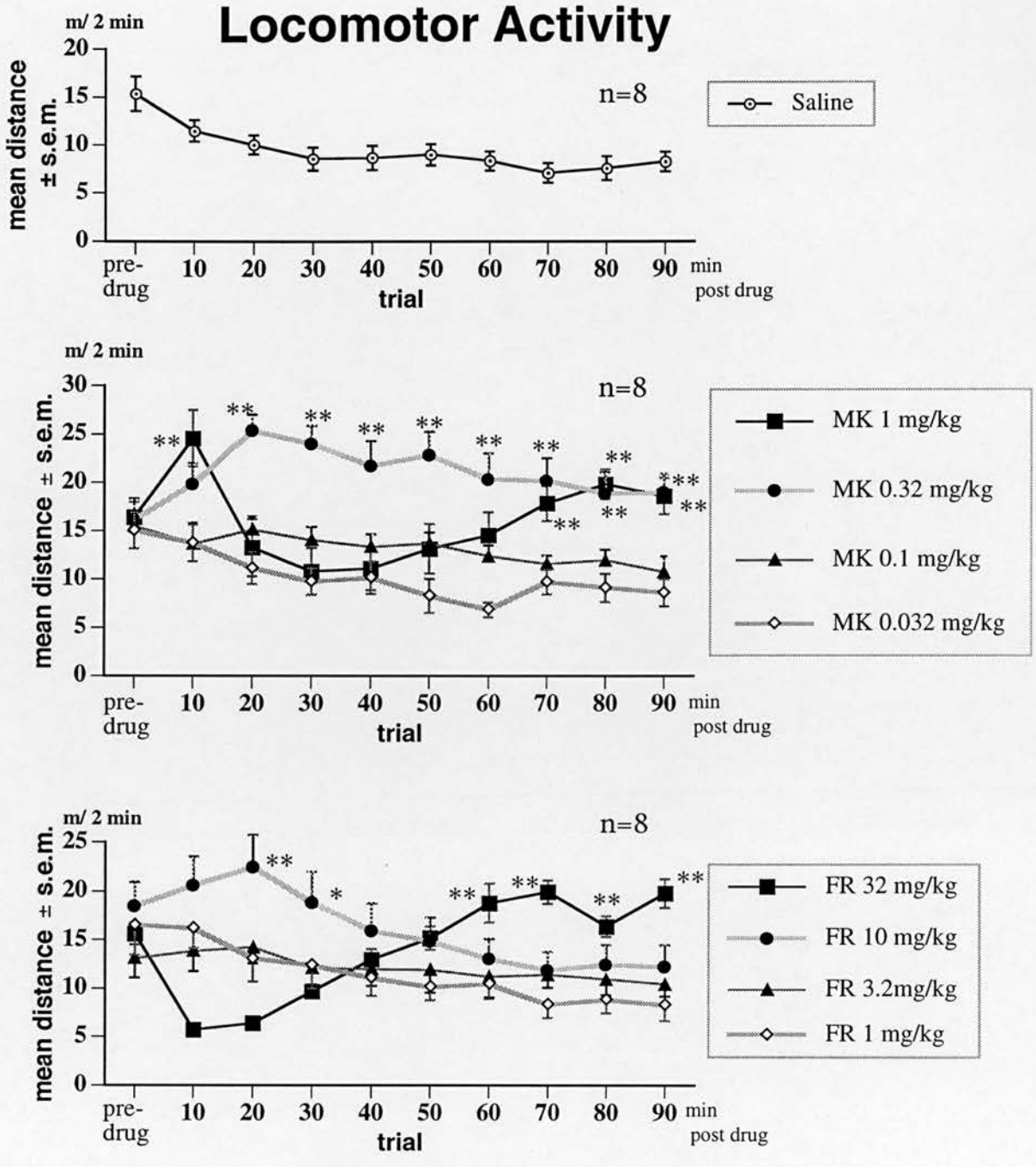
Fig. 3-4 shows the locomotor activity expressed by the mean (± s.e.m.) distance which an animal traversed across the test stage during each two min trial. As with other indices, an overall one-way between- and within-subjects ANOVA of locomotor activity scores revealed significant effects of group ($p < 0.0001$), trial ($p < 0.0001$) and group x trial interaction ($p < 0.0001$) (Table 3-1).

The time course of locomotor activity of the saline group shows a typical habituation pattern in which the activity decreased with the repetition of test trial and reached a stable level. One-way within-subjects ANOVA across the trials in the saline group detects this change as a significant effect of trial [$F(9, 63)=5.434$ $p < 0.0001$] (Table 3-5). Dunnett's pairwise comparison test indicates that the locomotor activity at 20 min post-injection trial and the later trials (20 min ~ 90 min) was significantly lower than at the initial pre-injection trial (pre-injection vs. 20 min, $p < 0.05$; pre-injection vs. 30 min or later trials, $p < 0.01$) (Table 3-5).

One-way between-subjects ANOVA followed by Dunnett's multiple comparison test was carried out to compare the locomotor activity of the saline group and drug groups (Table 3-6). The 1 mg/kg dose of MK-801 significantly increased locomotor activity at 10, 70, 80 and 90 min in comparison with saline ($p < 0.01$). Between 20 min and 60 min, the ataxic effect competed with the locomotor stimulating effect. 0.32 mg/kg MK-801 significantly increased locomotor activity at all post injection periods ($p < 0.05$ at 10 min , $p < 0.01$ between 20 and 90 min). The mean scores of locomotor activity of 0.1 mg/kg MK-801 group are not significantly

different from that of the saline group at any test trial. However, the 'habituation pattern', which is the significant decrease of locomotor activity in one-way within subjects ANOVA, was not detected in this group (table 3-5). This relatively higher locomotor activity in the late phase trials suggests that some kind of change in behaviour was induced after administration of 0.1 mg/kg of MK-801. Locomotor activity of 0.032 mg/kg MK group was not different from that of saline (Table 3-6). One-way within subjects ANOVA followed by Dunnett's test (Table 3-5) revealed a significant decrease of locomotor activity at 50, 60 and 90 min post drug periods in comparison with locomotor activity at the pre-injection period.

In the 32 mg/kg FR115427 group, locomotor activity was strongly suppressed between 10 and 30 min post drug periods by ataxia (Fig. 3-4). One-way within-subjects ANOVA followed by Dunnett's comparison test suggests the significance ($p < 0.01$) of this suppression (Table 3-5) . At 60 min or later, a significant increase ($p < 0.01$) of locomotor activity in this group in comparison with saline group is revealed by one-way between-subjects ANOVA and post hoc Dunnett's comparison test (Table 3-6, Fig. 3-4). The 10 mg/kg dose of FR significantly increased locomotor activity between 10 min and 30 min ($p < 0.05$ at 10 min, $p < 0.01$ at 20 and 30 min Table 3-6). As for 3.2 mg/kg FR group, one-way between subjects ANOVA (Table 3-6) did not detect any significant difference against saline group throughout the experimental period. However, one-way within-subjects ANOVA in 3.2 mg/kg group (Table 3-5) revealed no significant decrease of locomotor activity ('habituation pattern') which suggests some kind of change in the animals' behaviour. Locomotor activity of 1 mg/kg FR group was not significantly different from that of the saline group. One-way within-subjects ANOVA (Table 3-5) revealed a significant effect of trial ($p < 0.01$). Post hoc Dunnett's test showed the scores at 70, 80 and 90 min are significantly lower than the score of pre-injection period ($p < 0.05$ or 0.01).



** : $p < 0.01$ compared to saline group (Dunnett comparisonTest)

* : $p < 0.05$ compared to saline group (Dunnett comparisonTest)

Fig. 3-4 Comparison of locomotor activity induced by saline MK-801 and FR115427 in rats. The rating scale was the distance that an animal traversed during the 2 min test trial. Values are the means ± s.e.m. of eight animals

Table 3-5

One-way within-subjects ANOVA

Locomotor Activity

Group	saline	MK 1.0	MK 0.32	MK 0.1	MK 0.032	FR 32	FR 10	FR 3.2	FR 1.0
Trial Effect F(9,63)	5.434	7.108	2.739	1.454	3.301	22.278	4.029	0.954	3.387
Trial effect P value	p < 0.0001***	p < 0.0001***	p = 0.0092 **	p =0.1850	p = 0.0023 **	p < 0.0001***	p = 0.0004***	p = 0.4860	p = 0.0019 **
Dunnett Comparison test									
pre-drug vs. **10min** trial	n.s.	p<0.01 **	n.s.	-	n.s.	p<0.01 **	n.s.	-	n.s.
pre-drug vs. **20min** trial	p<0.01 **	n.s.	p<0.01 **	-	n.s.	p<0.01 **	n.s.	-	n.s.
pre-drug vs. **30min** trial	p<0.01 **	n.s.	p<0.01 **	-	n.s.	p<0.01 **	n.s.	-	n.s.
pre-drug vs. **40min** trial	p<0.01 **	n.s.	n.s.	-	n.s.	n.s.	n.s.	-	n.s.
pre-drug vs. **50min** trial	p<0.01 **	n.s.	p<0.05 *	-	p<0.01 **	n.s.	n.s.	-	n.s.
pre-drug vs. **60min** trial	p<0.01 **	n.s.	n.s.	-	p<0.01 **	n.s.	n.s.	-	n.s.
pre-drug vs. **70min** trial	p<0.01 **	n.s.	n.s.	-	n.s.	n.s.	n.s.	-	p<0.01 **
pre-drug vs. **80min** trial	p<0.01 **	n.s.	n.s.	-	p<0.05 *	n.s.	n.s.	-	p<0.05 *
pre-drug vs. **90min** trial	p<0.01 **	n.s.	n.s.	-	p<0.05 *	n.s.	n.s.	-	p<0.01 **

- : test is not carried out because trial effect is not significant

n.s. : not significant

Table 3-6

One-way between-subjects ANOVA

Locomotor Activity

Trial (min post drug)	pre-drug	10	20	30	40	50	60	70	80	90
Group effect F(8,63)	0.498	6.920	8.712	6.680	4.036	5.194	5.685	10.544	8.143	8.831
Group effect P value	0.8529	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.001	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001
Dunnett Multiple Comparison test										
Saline vs. **MK 1.0** mg/kg	-	p<0.01 **	n.s.	n.s.	n.s.	n.s.	n.s.	p<0.01 **	p<0.01 **	p<0.01 **
Saline vs. **MK 0.32** mg/kg	-	p<0.05 *	p<0.01 **	p<0.01 **	p<0.01 **	p<0.01 **	p<0.01 **	p<0.01 **	p<0.01 **	p<0.01 **
Saline vs. **MK 0.1** mg/kg	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Saline vs. **MK0.032** mg/kg	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Saline vs. **FR 32** mg/kg	-	n.s.	n.s.	n.s.	n.s.	n.s.	p<0.01 **	p<0.01 **	p<0.01 **	p<0.01 **
Saline vs. **FR 10** mg/kg	-	p<0.05 *	p<0.01 **	p<0.01 **	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Saline vs. **FR 3.2** mg/kg	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Saline vs. **FR 1.0** mg/kg	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

- : test is not carried out because group effect is not significant

n.s. : not significant

3.4 Discussion

The main findings of this experiment are:

(1) FR115427 as well as MK-801 induces stereotyped motor syndrome like ataxia, head weaving, body rolling and hyper locomotion.

(2) The potency of FR115427 to induce the above motor syndrome is about 30 times, or more, weaker than that of MK-801.

(3) The animals receiving FR115427 show a quicker onset of motor syndrome and quicker recovery from that effects than the animals receiving of MK-801. A peak effect of the ataxia and head weaving is induced within 30 min after injection of FR115427. The time course of locomotor activity and body rolling is complicated because of the ataxia.

3.4.1 Dose response of MK-801 and FR115427

The minimum effective doses of both drugs that induced significant changes in the behavioural indices in comparison with the saline group were as follows:

	Ataxia	Head weaving	Body rolling	Locomotor activity
MK-801 (mg/kg)	0.32	0.32	1.0	0.32
FR115427 (mg/kg)	32	10	32	10
approximate ratio	100	30	30	30

The absolute figures for minimum effective dose is variable according to the method of analysis. Some multiple comparison tests other than the Wilcoxon test may show different minimum effective doses. If the analysis is carried out across trials rather than across groups, different aspects of drugs effects are detected. In the analysis of locomotor activity, the one-way within-subjects ANOVA across trials revealed the disappearance of the saline group like habituation pattern in the locomotor activity at the dose of 0.1 mg/kg of MK-801 and 3.2 mg/kg of FR115427 (Table 3-5) (the approximate ratio of the potency was 30 to 1).

The sensitivity of these behavioural indices to detect the drug's effect is also different between experiments. Hiramats et al. (1989) observed ataxia with MK-801 at the dose of 0.25 mg/kg (i.p.), and increased head weaving and locomotor activity at 0.125 mg/kg (i.p.). Löscher and Hönack (1992) observed intense head weaving and hyper locomotion and moderate to marked ataxia at the dose of 0.1 mg/kg MK-801 (i.p.). Ouagazzal et al. reported the minimum effective dose of MK-801 to induce a significant increase in locomotor activity at 0.3 mg/kg (i.p.) in 1993 and 0.1 mg/kg (i.p.) in 1994. Thus, the reported behavioural effect of MK-801 in rats was observed at a little lower dose than in this experiment. The difference in the experimental conditions, especially any differences in the stress level of the animals during test trials, may modulate the potency of the drug's effect on the animal's behaviour. In this experiment, the animals were handled by the experimenter and received habituation trials beforehand to reduce stress of animals.

In any event, the extent of difference in potency of MK-801 between the present result and that found by others was less than 3 fold (There are no comparable data for body rolling). In this as well as in other experiments, the effective dose of MK-801 that induced ataxia, head weaving and locomotor stimulation was lower than the reported therapeutic doses required to block ischaemic brain damage (MK-801 reduced ischaemic brain damage at about 1 to 5 mg/kg i.p. in the rat middle

cerebral artery occlusion model (Dirnagle et al., 1990; Bielenberge and Beck, 1991; Gill et al., 1992; Iijima et al., 1992; Frazzini et al., 1994).

The above dose is also lower than the dose (1 mg/kg i.p.) at which MK-801 blocks the induction of LTP in anaesthetised rats (Abraham and Mason 1989). In other words, it is difficult to carry out a behavioural experiment at a dose at which MK-801 blocks the brain damage or the induction of LTP because of the ataxic effect.

The potency of FR115427 to induce behavioural effects was approximately 30 to 100 times weaker than the potency of MK-801. This difference in potency seems to be slightly greater than the difference in their binding affinity for the NMDA receptor in rat brain membranes. As described in Chapter 1, Hodgkiss et al. (1993) reported that FR115427 is 14-fold less potent than MK-801 as inhibitors of [^{3}H] MK-801 binding (the Ki values for FR115427 and MK-801 were 43.3 nM and 3.14 nM respectively in the presence of 10 μM L-glutamate). Sherriffs et al. (1993) reported that FR115427 was 10-fold less potent than MK-801 as inhibitors of [^{3}H] MK-801 binding (the Ki values for FR115427 and MK-801 were 35.4 nM and 3.57 nM respectively in the presence of 10 μM L-glutamate).

The weaker *in vivo* behavioural effects of FR115427 relative to its affinity for the NMDA receptor does not seem to be explained by lower absorption or distribution of this compound in the brain after systemic administration because, in an NMDA-induced convulsion test in mice conducted by Nakanishi et al. (1995), FR115427 was found to be about 10 times less potent than MK-801 by three different routes of administration (i.c.v., i.p. and p.o.) In the MCA occlusion ischaemia model in rat, the therapeutically effective dose of FR115427 (10 mg/kg i.p.) is also just about only 10 times higher than that of MK-801 (1 mg/kg i.p.).(Katsuta et al., 1995). These differences in potency between FR and MK are within the range of their difference in affinity for the receptor. A possible

explanation of the weaker behavioural effects of FR115427 lies in a differential requirement of L-glutamate or glycine in their binding to brain membrane because the *in vivo* models in which FR shows only 10 times weaker potency than MK-801 are expected to be accompanied with an elevated level of agonists for NMDA receptors (due to injection of NMDA or release of glutamate) in the brain. According to the Sherriffs et al.(1993), the specific binding of [^{3}H] FR115427 in the absence of exogenous L-glutamate was too low for exact kinetics while [^{3}H] MK-801 binding was measurable in the absence of added exogenous L-glutamate due to its higher affinity for the NMDA receptor ion channel. In another words, the pharmacologically effective binding of FR may be more greatly affected by the concentration of glutamate or another amino acid in the synaptic gap. As discussed in Chapter 1, the extracellular level of glutamate is suggested to be dynamically modulated in the brain and the binding of non-competitive NMDA antagonists could be affected by the level of glutamate. For example, *in vivo* binding of [^{3}H] MK-801 to the ischaemic cortex and striatum was found to be significantly enhanced in circumstances in which the extra cellular glutamate level was likely to be elevated (Wallace et al., 1992). The binding affinity FR115427 may be much lower than MK-801 if the extra cellular level of excitatory amino acids are very low or if amino acid uptake activity is very high.

The relatively low potency of FR115427 in inducing ataxia may be an advantage of this drcug as a pharmacological tool because the ataxic effect of non-competitive antagonist is the main problem in studying the effect of this type of drug on learning.

As up to 10 mg/kg FR115427 did not induce serious troubles in the motor ability of rats, this dose seems to be the maximum dose for water maze experiments while only 0.1 mg/kg of MK-801 seems to be the maximum for that learning test.

3.4.2 The effect of ataxia on the other behavioural indices

Before discussing the time course of the behavioural effects of MK-801 and FR115427, the following section will comment on ataxia whose presence complicates the analysis of the time course of the drug's effect. The ataxic effect inhibits the locomotion of animal which is the prerequisite for the expression of head weaving, body rolling and hyper locomotion. Therefore the analysis of the behavioural effects of MK-801 and FR115427 bears the paradoxical problem that one drug effect inhibits another effect of its own. At the top doses of both drugs, the above paradox severely distorted the time course of head weaving, body rolling and locomotor activity. In these indices, an increase in the scores seems to indicate the recovery from the ataxic effect rather than a real intensification of the drug's effect because (1) the peak of the scores appeared in the declining phase of ataxic score; (2) the peak of the scores of lower dose groups which suffered less ataxia appeared at earlier periods; (3) the fact that the recovery of the scores of head weaving was faster than the recovery of body rolling scores or locomotor activity in the top dose groups is consistent with the observation that the recovery of small head movements (necessary to express head weaving) was faster than the recovery of the activity of the whole body in ataxic animals.

In the discussion of time course of head weaving, body rolling and locomotor activity, the scores of lower dose groups seems more reliable than the scores of the top doses although the change in the scores of lower doses did not always induce statistically significant effects.

3.4.3 The time course of the behavioural effects

The ataxic effect of FR115427 was exhibited very quickly. The score for ataxia in the 32 mg/kg FR group reached its peak between 10 min and 30 min after injection and then decreased gradually. FR 10 mg/kg group showed a consistent time course of score. As the head movement of the animals recovered from ataxia relatively faster than the whole body locomotion, the score of head weaving of 32 mg/kg FR group showed a similar time course to ataxia except the slight delay of onset. The score of body rolling and locomotor activity of the highest dose group was severely distorted by the ataxic effect. The score of the lower dose group suggests a similar time course of onset and recovery to that of ataxia. That is, the peak effect of the motor syndrome in the FR group was induced during the first 30 min after injection then the effect steadily reduced by the 90 min post drug period.

The behavioural effect of MK-801 was induced more slowly and sustained for a longer period. A broad peak of score was observed between about 20 min and 60 min after the injection in the score for ataxia and head weaving at a dose of 1 mg/kg and 0.32 mg/kg, and in locomotor activity at the 0.32 mg/kg dose. The score for body rolling and locomotor activity of the top dose group reached peak at around 90 min or later.

3.4.4 Conclusion

The potency of FR115427 to induce a motor syndrome is about 30 to 100-fold weaker than that of MK-801. As the behavioural side effects of FR115427 seem to be weaker than other physiological effects of this compound, FR 115427 can be tested at a relatively higher dose in the water maze experiment. Because of the ataxic condition induced by MK-801 and FR115427, the maximum doses that can be tested seems to be 0.1 mg/kg for MK-801 and 10 mg/kg for FR115427. Since FR115427 induced maximum scores for the behavioural syndrome at about 20 to 30 min after injection, one condition of the training and testing of animals in the water maze is that it has to be carried out in this time window to examine the relationship between the effect of FR115427 on behaviour and on learning performance. However, this does not preclude the use of later post-injection time-periods if the electrophysiological studies (Chapter 4) indicate that their examination would be profitable. As the effect of MK-801 was induced slowly and sustained for a long time, it is difficult to discuss the time dependence of the effect of MK-801.

Chapter 4
Experiment 2: In vivo LTP study (1)

4.1 Introduction

Some investigations have found that MK-801 impairs the acquisition of learning in the water maze but not the retention of the acquired performance, and they have related the impairment to the inhibitory effect on LTP exhibited by NMDA antagonists which, by analogy, block its induction but not expression (Robinson et al., 1989, Heale and Harley 1990). However, they have not actually tested the effect of MK-801 on the induction of LTP in vivo. This seems to be partly because they cannot test MK-801 in the water maze at the consistent dose range at which MK-801 has a detectable effect on LTP because of the strong ataxic effect of MK-801.

The behavioural test in Chapter 3 revealed that the ataxic effect of FR115427 was about 30 to 100 times weaker than that of MK-801. This finding suggests that FR115427 can be a good substitute for MK-801 to investigate the effect of a non-competitive NMDA antagonist on spatial learning and LTP at the same dose range.

The purpose of the investigation in this chapter was:

(1) to find the conditions in which systemically administrated FR115427 blocks the induction of LTP in hippocampus;

(2) to examine whether the effect of FR115427 on LTP is exhibited across a similar time course to that observed in the behavioural experiments in Chapter 3.

The time course of the drug's effect on LTP induction was examined by modulating the interval between drug administration and the application of tetanus stimulation. In this experiment, two different intervals (short and long) were arranged to see whether the effect is an early onset type or late onset type.

The general experimental procedure was described in Chapter 2.

4.2 The effect of FR115427 after various intervals

4.2.1 Method

In order to find the suitable intervals between drug treatment and induction of LTP for the following experiment, tetanus stimulation of perforant path fibres was applied 20, 30, 90 and 150 min following i.p. injection of 10 mg/kg dose of FR115427 (in 0.9 % saline). During electrophysiological recordings, 4 animals of the 18 animals receiving 10 mg/kg FR injection died. The data from 14 surviving animals were adopted for analysis (Data were sometimes collected from both hemispheres of an individual rat).

The extent of potentiation of EPSP was assessed by the increase of EPSP slope (Fig. 4-1). The increase of slope was expressed by the % increase in the mean slope at 30 min after tetanus (actually the mean for 5 min between 30 and 35 min after tetanus) in comparison with the mean slope just before tetanus (mean for 5 min period).

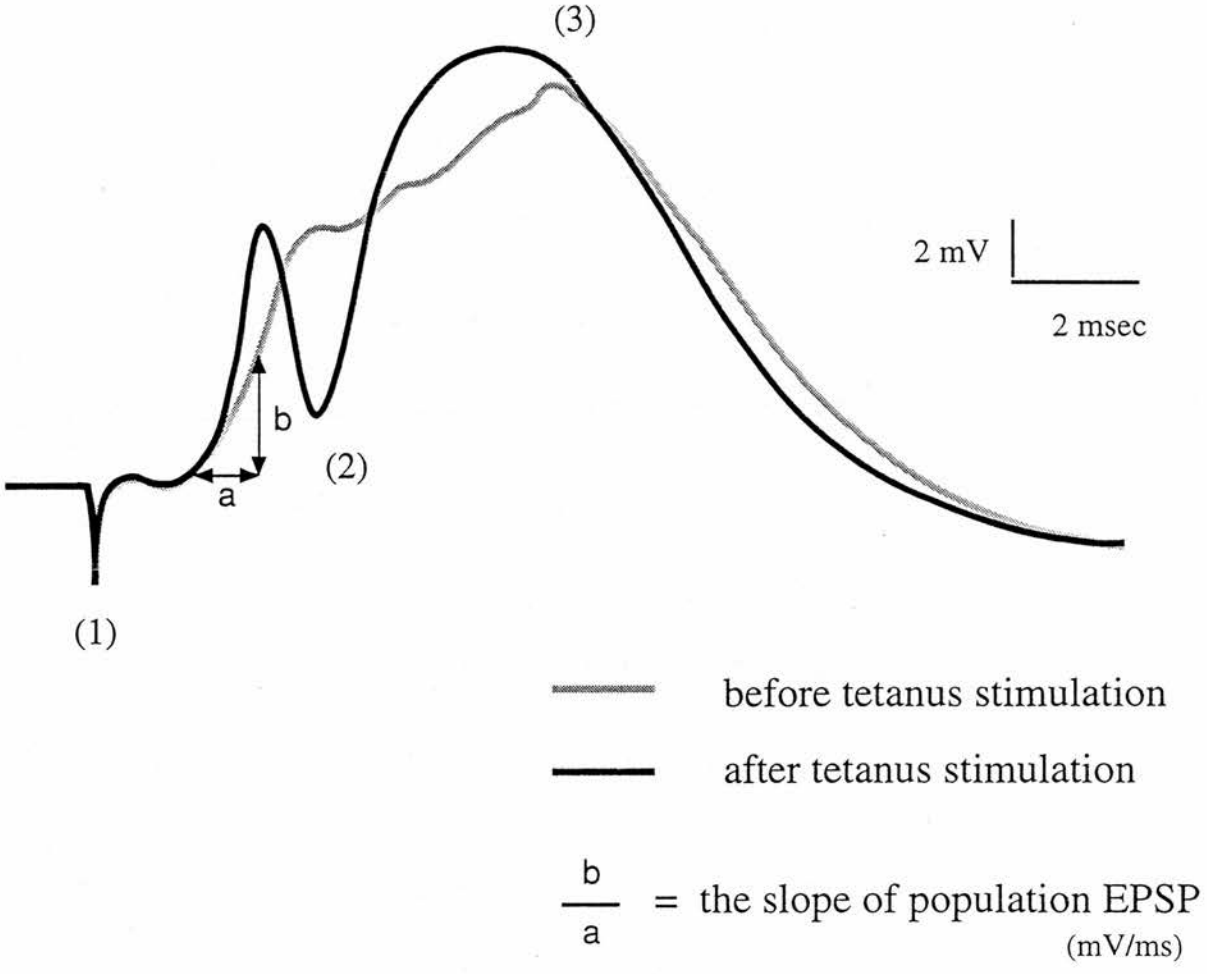


Fig. 4-1

Typical evoked population EPSPs in the dentate gyrus recorded just before and 1 min after tetanus stimulation. Recordings are from from a single rat injected with saline.

Marked potentiation of the slope of the population epsp (b/a) was observed after tetanus stimulation. (1) The stimulus artifact, (2) The negative-going population spike and (3) The positive going epsp are shown

4.2.2 Results

Fig. 4-2 shows the % increase of epsp slope induced by tetanus applied after various interval following i.p. injection of 10 mg/kg FR 115427. Although ANOVA on a percentage change did not indicate significant group effect [$F(3,14) = 2.555$ $p = 0.097$], strongest suppression of slope increase was observed 90 min after FR injection and weakest effect was observed 30 min after FR injection.

4.2.3 Discussion

The comparison of the results between short intervals (20 or 30 min) and long intervals (90 or 150 min) suggested the late onset effect of FR on LTP. This time course of drug's effect seemed to be in contrast to the effect on behaviour shown in Chapter 3. In order to confirm this time dependent manner of the effect on LTP, a dose-response study was carried out with short interval and long interval. As the short interval, 30 min was used for the following study because a 30 min interval allows sufficient time to get a stable baseline of the EPSP slope (the amplitude of EPSP sometimes fluctuated just after drug administration). In the later water maze experiments, it is impossible to assess the drug's effect at an exact time point since one day's training (6 trials) takes more than 10 min for each animal. If there were not any essential differences between the result of 20 min interval and 30 min interval LTP experiment, both experiments can be a reference for the water maze experiment with 20 min interval between drug injection and training. Because the strongest suppression of slope increase was observed at 90 min, this interval was chosen for further analysis in the dose response study.

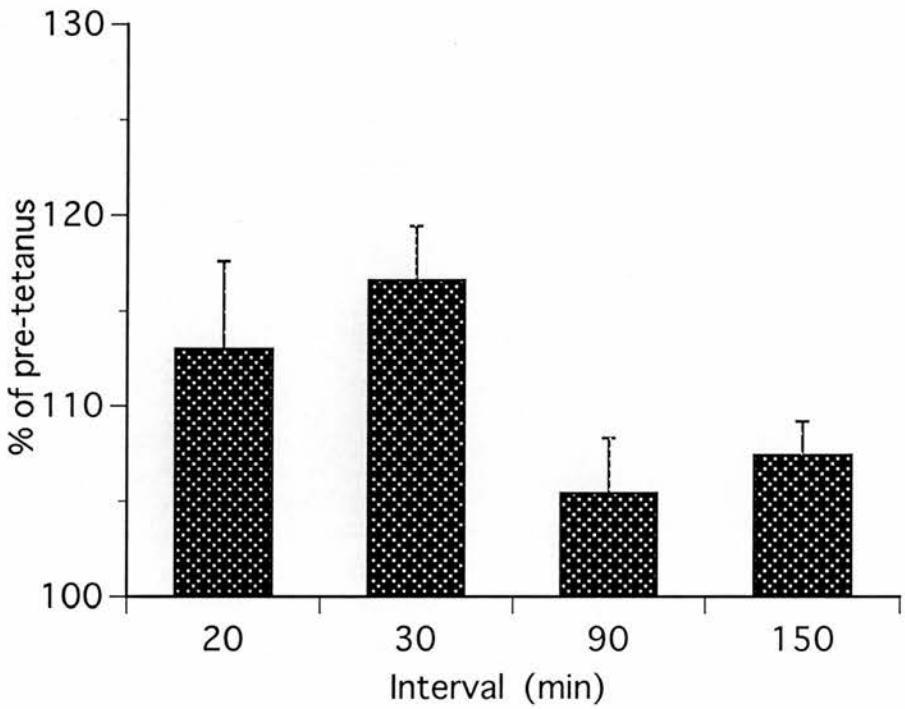


Fig. 4-2 Mean (+s.e.m.) % slope epsp after potentiation
Effect of 10 mg/kg of FR115427 on LTP induced after various interval

4.3 Dose-response study

4.3.1 Method

Rats were anaesthetised with Urethane (1.5 g/kg i.p.) and placed in a electrophysiological recording system as described in Chapter 2.

A single i.p. injection of saline (1 ml/kg), MK-801(0.1, 0.32, 1.0 mg/kg) or FR115427 (1.0, 3.2, 10.0 mg/kg) was made for each animal after 10 min baseline of recording. Tetanus stimulation was given at either 30 or 90 min intervals after drug administration. The results with the 30 and 90 min intervals of the pilot experiment (above) were combined with the results of the 10 mg/kg FR group.

Unfortunately, 5 animals out of 5 which received 1 mg/kg MK-801 died. Therefore the experiment at this dose was not carried out further. One animal out of 5 that received 0.32 mg/kg MK-801 died. One animal out of 6 which received 3.2 mg/kg FR115427 died As described above, 4 out of 18 died in the 10 mg/kg FR group. All animals which received 0.1 mg/kg MK-801 or 1 mg/kg FR115427 survived until the end of recording. Only the results from the surviving animals were used for analysis. In the end, there were 12 different experimental groups in this experiment (6 different drug treatment for each interval test).

Fig. 4-3 shows an example of EPSP slope recorded every 20 sec in a control experiment (the values were normalized and averaged). To assess the effects of drug on baseline, the average slope value between 25 min and 30 min (15 data points) after drug injection ('pre-tetanus period' in Fig. 4-3) were made and compared to the average value obtained just prior to drug injection period ('pre-drug period' in Fig. 4-3). To assess the elicited percentage change in EPSP by tetanus stimulation, averages of slope value from 15 wave forms (5 min 'LTP period' in Fig. 4-3) 30 min

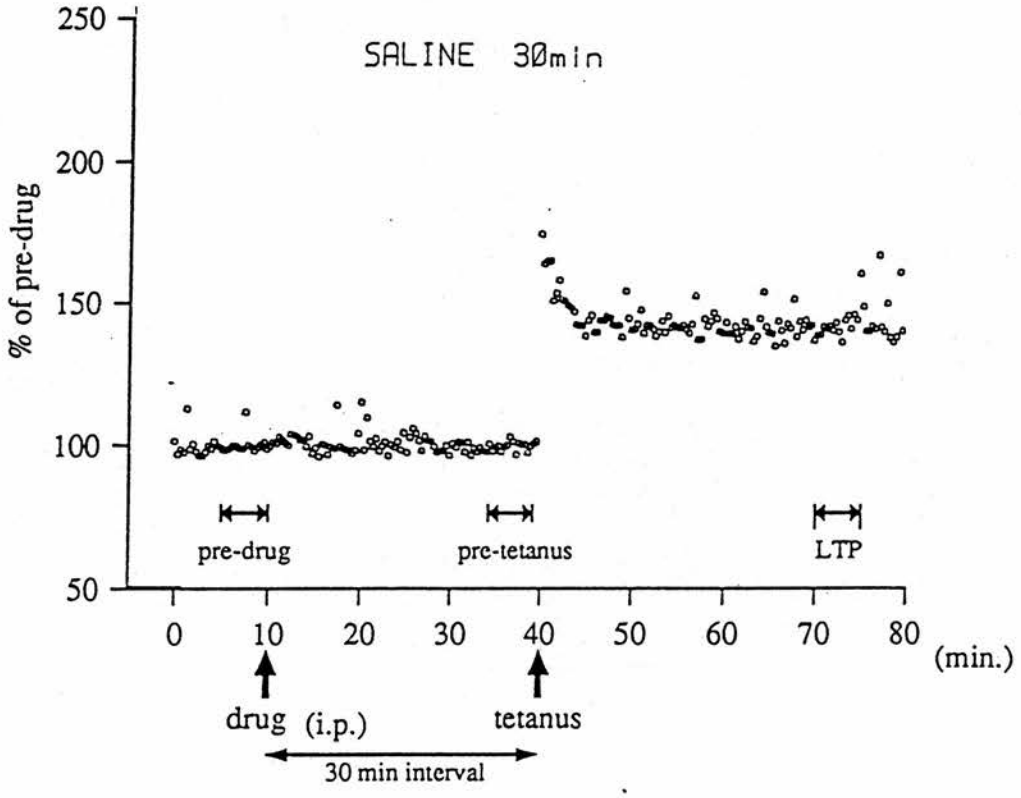


Fig. 4-3 Time plot of epsp slope from saline 30 min group (n=5). Values were averaged and normalized (pre-drug period = 100)

after tetanus stimulation were made and compared with the average just before tetanus stimulation ("pre-tetanus' period).

4.3.2 Results

(a) Drug's effect on baseline

The effect of drug dose on baseline was investigated in 30 min interval experiments. The absolute value of slope EPSP recorded before the drug treatments was in the range of 1.9 ~ 5.5 mV/ms. All groups showed a small increase in the EPSP slope after drug treatment (Fig. 4-4). While an ANOVA of % change across 6 drug groups did not indicate significant group effect [$F(5,21) = 1.676$ $p = 0.184$], one-way within-subjects ANOVA for individual groups, which was carried out to compare the values before and after (25 ~ 30 min) drug treatment, revealed that MK-801 induced a significant increase in slope value against pre-drug period at two doses (MK 0.1 mg/kg group; [$F(3,1) = 10.429$ $p < 0.05$], 0.32 mg/kg group;[$F(3,1) = 48.375$ $p < 0.01$]). No significant increase in slope value was detected in saline and FR groups.

(b) Drug's effect on LTP

The potentiation of slope EPSP induced by tetanus stimulation was suppressed by FR115427 in a dose and time dependent manner while the effect of MK-801 was equivocal at the tested doses (Fig. 4-5).

In order to analyse the time dependency of FR's effect independently, a two-way between-subjects ANOVA was performed across only FR treated groups. This analysis revealed a significant effect of interval [$F(1,22) = 11.312$ $p < 0.01$] and dose [$F(2,22) = 11.206$ $p < 0.001$]. The lack of any significant interaction term

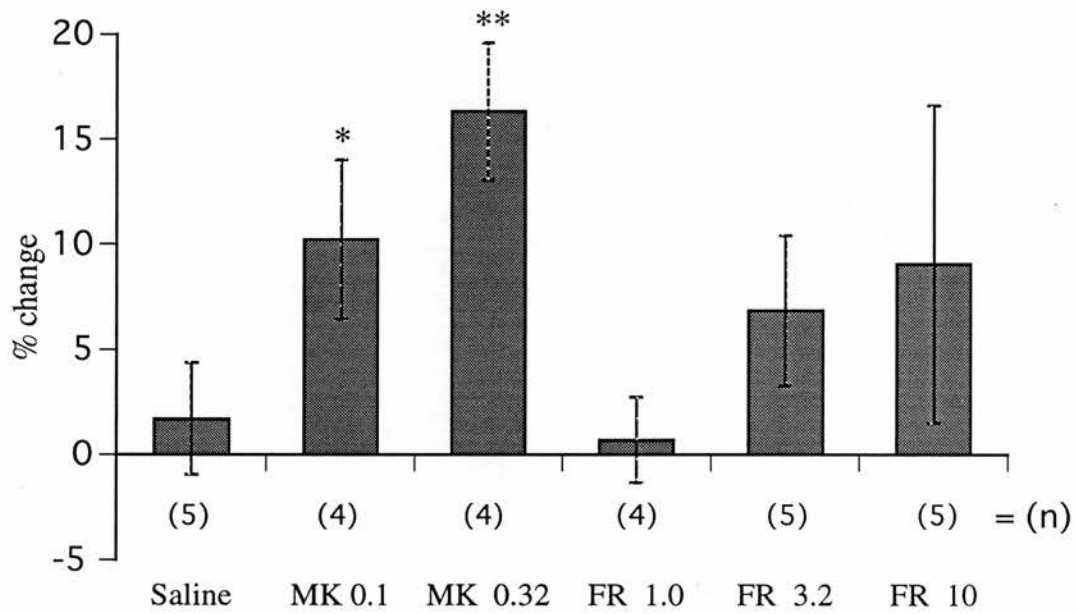


Fig. 4-4 % change in baseline of EPSP slope (mean ± s.e.m.) 25~30 min after drug injection

* : $p < 0.05$, ** : $p < 0.01$ before v.s. after drug treatment (one-way within-subjects ANOVA)

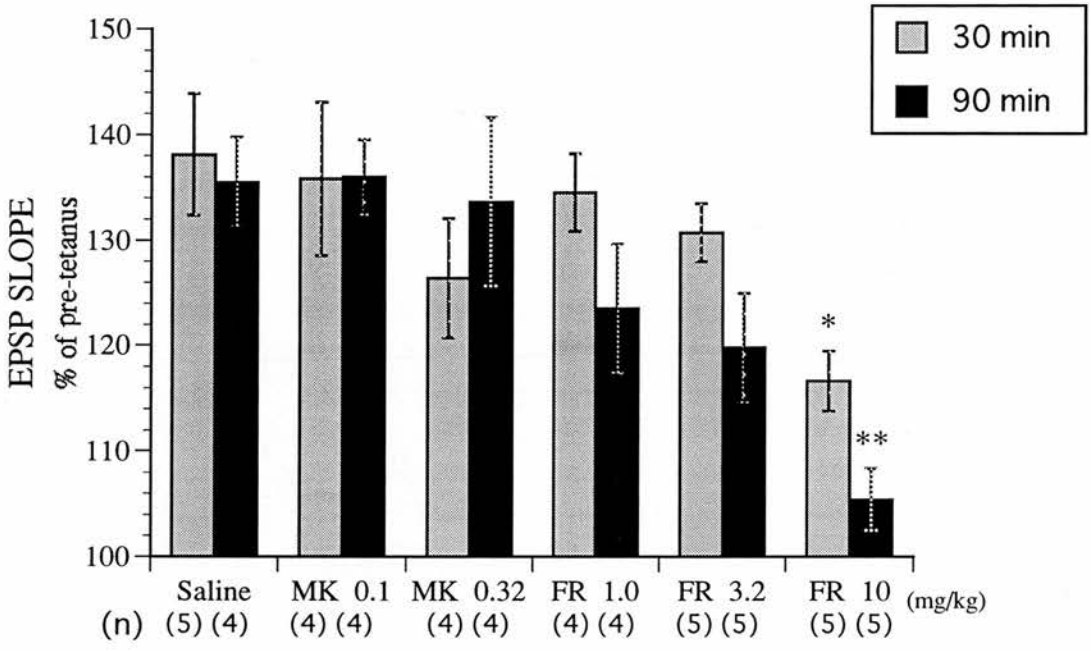


Fig. 4-5

Effect of FR115427 and MK-801 on LTP induction

mean % change (±s.e.m.) in epsp slope induced by tetanus stimulation 30 min (shaded bars) or 90 min (filled bars) after injection (i.p.)
values are means for 5 min between 30 min and 35 min after tetenus.
* : $p < 0.05$ ** : $p < 0.01$
significant difference from corresponding saline control group
(Dunnett multiple comparison test)

(interval x drug dose) [$F(1, 22) < 1$], statistically confirms the tendency that 90 min effect of FR was greater than 30 min effect at all doses. Tukey follow-up comparisons among groups indicated the effect of 10 mg/kg (showing only 11.0% LTP) was significantly more potent in suppressing LTP than that of 1 mg/kg (29% LTP) ($p < 0.01$) and 3.2 mg/kg (25.2% LTP was induced) ($p < 0.01$) (This is the comparison between groups including both 30 min and 90 min because of the constitution of the two-way analysis : the % figures are the averages of 30 min and 90 min experiments). A comparison between the effect after 30 min interval and 90 min interval confirmed that the effect of FR 90 min after injection (16.2 % potentiation) was significantly more potent than 30 min after injection (27.3% potentiation) in suppressing LTP (Tukey $p < 0.01$).

Following the above two-way analysis indicating that the effect of FR was time dependent, one-way between-subjects ANOVAs were carried out on the results of the 30 min interval experiment and the 90 min interval experiments separately, which allows comparison between control saline group and a drug group.

The ANOVA on the 30 min experimental groups revealed a significant effect of group [$F(5,21) = .719$ $p < 0.05$)]. A Dunnett post hoc comparison indicated a significant difference between control group (38.1% potentiation) and FR 10 mg/kg group (16.6% potentiation) ($p < 0.05$). Therefore 10 mg/kg of FR significantly reduced LTP by 56.4% (38.1-16.6 / 38.1 x 100 %).

The analysis on the data in 90 min interval experiments revealed a significant effect of group [$F(5,20) = 5.359$ $p < 0.01$]. A Dunnett multiple comparison test indicated a significant difference between control group (35.5% potentiation) and FR 10 mg/kg group (5.4% potentiation) ($p<0.01$). Therefore 10 mg/kg of FR significantly reduced LTP by 84.9% (Fig.4-5). Fig. 4-6 shows a plot of the averaged EPSP slope indicating that induction of LTP was effectively blocked by 10 mg/kg of FR115427 administered 90 min before tetanus.

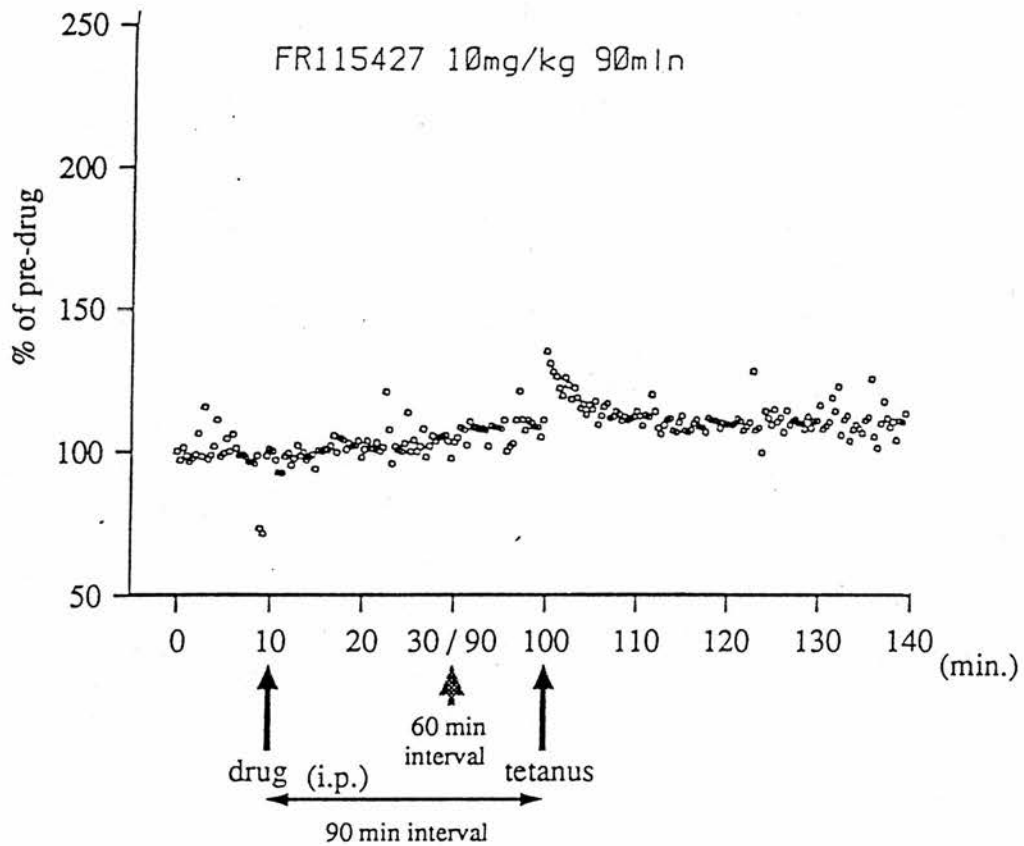


Fig. 4-6 Time plot of epsp slope from FR 10 mg/kg 90 min group (n=5). Values were averaged and normalized (pre-drug period = 100) 20 min after the drug injection, 60 min interval was taken then recording was restarted

After the ANOVA across groups to analyse the drug dose effect and interval effect, a separate analysis was carried out on the result of each group to examine the effect of tetanus stimulation on EPSP slope. A one-way within-subjects ANOVA (slope value just before tetanus vs. after tetanus) revealed that the tetanus stimulation induced a significant increase in slope value in all groups except the FR 10 mg/kg 90 min group at the level of $p < 0.05$ or 0.01. As shown in Fig.4-5 and Fig. 4-6, tetanus stimulation did not induce a significant change in slope value in the FR 10 mg/kg 90 min group [$(F(4,1) = 4.239 \quad p = 0.109]$. In another words, 10 mg/kg of FR115427 blocked induction of LTP when the tetanus stimulation was applied 90 min after i.p. injection.

4.3.3 Discussion

(a) Toxicity

The toxic effects of FR115427 and MK-801 seemed to be synergistic with urethane, because injection of FR or MK alone had never caused an animal's death in any other experiment. Single i.p. injection of up to 1 mg/kg MK or 32 mg/kg FR was not lethal in the Experiment 1 (the open field activity test). Similarly, multiple injections (5 consecutive daily injections) of up to 0.32 mg/kg of MK or 10 mg/kg of FR in the Experiment 4 (the water maze experiment) did not cause death either.

Abraham and Mason.(1988) or Morimoto et al.(1991) did not report any death caused by i.p. injection of 1 mg/kg or 2 mg/kg of MK-801 in urethane (1.2~1.5 g/kg i.p.) anaesthetised rat. The difference is possibly due to the difference in strain of animal; they used male Sprague-Dawley rats instead of Lister hooded rats.

After the injection of FR or MK, no particular pattern of change in the evoked EPSP was observed and it was always stable until just before the death. The loss of

the potential was sudden, about 30 to 60 min after drug injection. The mechanism of this toxicity is not clear.

(b) Effect on the baseline EPSP slope

The 0.1 and 0.32 mg/kg doses of MK-801 induced a small but significant increase in slope of EPSPs within 30 min. This change is possibly not caused by a change in body temperature because rectal temperature was monitored and maintained at a stable level (± 0.1°C) throughout the experiment (The body temperature was initially in the range of 36.1 ~ 36.8°C and the initial temperature was maintained throughout the experiment). Although a slight increase in slope EPSP was also observed in some animals which received FR115427, this effect was not statistically significant. In another published reports, the change in basal EPSP caused by MK-801 was not significant. Abraham and Mason (1988) reported a small but non-significant change in slope of the population EPSP in dentate gyrus 30 min after i.p. injection of 0.1 or 0.5 mg/kg of MK-801 (7.8% decrease or 0.7 % increase respectively). Manfred et al. (1993) reported a small but not significant decrease (less than 10%) in slope of the population EPSP in the dentate gyrus of freely moving rats 30 min after i.p. injection of 0.2 mg/kg MK-801. The variance of the results between the experiments suggested that the slight effect of MK-801 was not exhibited by the direct inhibition of neurotransmission in the hippocampus but by some indirect physiological change such as blood pressure or energy supply, which could be sensitive to minor modification of experimental conditions across laboratories. MK-801 (0.3 mg/kg i.v.) induces a much stronger increase in local cerebral glucose utilisation in limbic system than FR (3 mg/kg i.v.) (personal communication with Sharkey, J.). This effect may partly explain the stronger effect of MK-801 on EPSP slope in this experiment.

In any event, MK-801 and FR115427 did not block or depress synaptic transmission evoked by low frequency stimulation of the principal afferent fibres.

(c) Effect on LTP

The dose dependent and time dependent effect of FR115427 on LTP was similar to the effect of MK-801 reported by Abraham and Mason (1988) who found that LTP was only blocked when the tetanus was given 150 min after 1 mg/kg i.p. administration. This effect is consistent with the idea that FR115427 acts as a non-competitive NMDA receptor antagonist *in vivo*. The potency of FR's effect on LTP was about ten times weaker than that of MK-801 reported by Abraham and Mason. This ratio is roughly consistent with the ratio in the affinity to the MK-801 binding site (Sherriffs et al., 1993, Hodgkiss et al., 1993) and the therapeutic potency in the MCA occlusion model (Katsuta et al., 1995). The relative potency of FR115427 in inhibiting LTP was stronger than that in the induction of the motor syndrome (FR was found to be 30 to 100 times weaker than MK-801 in Experiment 1). As discussed in Chapter 3, the enhanced glutamate release during the tetanus stimulation could increase the potency of FR115427. The relatively strong effect of FR115427 on LTP in comparison with the motor side effects is an advantage of this drug as a pharmacological tool.

Although the inhibitory effect of 1 mg/kg MK-801 on LTP could not be replicated in the present experiment because of toxicity to anaesthetised Lister hooded rats, Morimoto et al. (1991) confirmed that 1 and 2 mg/kg of MK-801 almost completely blocked LTP of the population EPSP induced 120 min after i.p administration in anaesthetised SD rats. At the lower dose of MK-801, neither Abraham and Mason (0.5 or 0.1 mg/kg) nor the present experiments, could detect any significant effect on EPSP potentiation.

The mechanism of enhancement of the effectiveness of peripherally administered FR115427 and MK-801 in suppressing LTP after long interval (90 to 150 min)is unclear. The "use-dependent" effectiveness of the non-competitive NMDA antagonists (Church et al, 1987; Kemp et al., 1986) possibly explains this long latency. According to this idea, the NMDA receptor may be spontaneously activated *in vivo* during 90 or 150 min interval allowing FR115427 or MK-801 access to their binding site. In favour of this idea, Abraham and Kairiss (1988) found AP5 sensitive (NMDA receptor mediated) spontaneous complex spike firing by CA1 neurons in urethane-anaesthetised animals. However, it is difficult to examine whether that slow development of LTP inhibition depends on the spontaneous glutamate release *in vivo* (Abraham and Mason noted that development was at least, independent of the stimulus test current). There is a possibility that FR115427 or MK-801 slowly access their binding site by diffusing in the membrane lipid other than through the ion channel of the receptor.

In any event, it is interesting that the time course of the effect of FR115427 on hippocampal LTP (late onset) and on stereotyped behaviour (early onset) had a clear contrast. Probably, this difference is explained by the difference of the brain region where those effects are exhibited. FR115427 may act in a variety of kinetics modes according to a level of extracellular glutamate or a firing rate of neurons or a distribution of receptor subtypes or unknown factors in the various part of the brain.

The following water maze experiments make the most of the pharmacological characteristics of FR115427: the clear contrast in the time course between the behavioural effects and electrophysiological effects. In order to examine whether any drug effect on learning is related to the effect on hippocampal LTP or another

behavioural effect, water maze training was carried not only 20 ~30 min after drug administration, but also 90 ~ 100 min after injection. In order to examine whether LTP represents the synaptic plasticity associated with spatial memory acquisition, up to 10 mg/kg of FR115427 was tested in the water maze.

Chapter 5
Experiment 3: Hippocampal Lesion Study

5.1 Introduction

Before proceeding to examine the effect of FR115427 on spatial learning, animals with whole hippocampal lesions were tested in the water maze using an almost identical procedure to that to be reported later using the drug (Chapter 6). This lesion study examined whether spatial learning (place navigation) in the water maze is sensitive to damage in the hippocampus. This point is important for the following discussion about the relationship between the drug's effect on hippocampal function (LTP) and its effect on spatial learning.

5.2 Methods

(a) Handling and pretraining trials

On the first week, 12 animals received 3 min handling for 3 days and 60 sec x 2 free swimming trials . The temperature of the milky water was adjusted to 25.0 ± 0.5 °C. The purpose of this handling and pretraining is to habituate the animals to the experimenter's handling and to the water maze environment.

(b) Lesion surgery

On the next week, 6 of the pretrained animals received bilateral complete hippocampal lesion surgery and the other 6 animals received sham surgery. The procedure of hippocampal lesion surgery described by Jarrard (1989) was modified in this experiment. Hippocampal lesions (HL) were made by 12 injections of ibotenic acid on each side of the hippocampus. Sham surgery involved inserting the injection into the overlying cortex only. One or two lesions and another one or two sham lesioning were carried out each day. It took 5 days to finish the surgery on all 12 animals. Animals were given a period of 16 to 21 days before commencing place navigation training to obtain substantial cell death in hippocampus and recovery from the acute effect of surgery.

(c) Place navigation training and transfer test

Three of the sham animals and 3 HL animals were trained to locate the submerged platform fixed in the NE quadrant and the other 3 sham and 3 HL animals were trained to locate the submerged platform fixed in the SW quadrant of the pool. Each animal received 6 trials a day for 4 days (Day 1 ~ Day 4). The escape latency on each trial was measured and recorded. If the rat failed to find the platform within 60 sec, it was guided to it by the experimenter (on such occasions, the escape latency was recorded as 60 sec). As the drugs to be used in the following experiments are expected to induce hypothermia, the maximum swimming time (60 sec) was shorter than in other reported experiments.

On Day 5, the animals were tested in the transfer test to assess their memory of the location of the submerged platform. In this test, the escape platform was removed from the pool and the animals was made to swim for 60 sec. The time spent in each quadrant of the pool was measured and analysed.

The temperature of water was kept at 27.0 ± 0.5°C during the training on Day 1. On Day 2 and the following day, the water temperature was kept at 25.0 ± 0.5°C. This modification of water temperature was made for the same reason as used to set the maximum swimming time.

(d) Cue navigation training

Immediately following the transfer test, the animals received six trials of additional training to learn to escape to a visible (cue) platform. This training was carried out to assess the motor ability and motivation of the animals to escape to the platform. Escape latency of each trial was measured and analysed.

None of the animals received any drug treatment during above training and testing in this experiment.

(e) Histology

The following week, animals were injected (i.p.) with a lethal overdose of Nembutal and transcardially perfused with saline followed by 10 % formalin. At this point, 3 or 4 weeks had passed since the injection of ibotenic acid into the hippocampus.

30 μm horizontal sections of brain were stained with fast cresyl violet and examined microscopically to assess the neuronal loss.

(f) Coordination of time schedule with Experiment 4

In the above schedule, the procedure of training and testing in the water maze was identical to that to be reported later using FR115427 (Experiment 4) except for the interval between pretraining trials and the main acquisition training. In this hippocampal lesion experiment (Experiment 3), there was a 2 ~ 3 week interval, in contrast to a 3 day interval in Experiment 4. Coordination of this interval can be

achieved by carrying out pretraining after lesion surgery. However, if the pretraining was carried out after lesion surgery, the extent of habituation to the training environment of HL animals may be different from that of sham animals. This experiment took priority for unification of the condition between sham animals and HL animals in the acquisition training.

5.3 Results

5.3.1 Histology

The examples of lesioned brain is shown in Fig. 5-1*. The result of the assessment of cell loss in hippocampus is shown in Table 5-1. About 90 % of the cells in hippocampus were removed by surgery. In most animals, the entorhinal cortex was completely left intact and the subiculum was fairly intact. Although some part of ventral hippocampus in some animals were spared, the network of whole hippocampus seems to be effectively destroyed because the whole parts throughout dentate gyrus to CA1 are not spared at any level of hippocampus. (Although a fair proportion of CA3 or CA1 neuron are spared in the most ventral level of hippocampus of No.3164, more than 50 % of granule cells in dentate gyrus were damaged.) Therefore the data of all hippocampal lesioned (HL) animals are included in the following analysis.

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* next page

Fig.5-1 Photographs of horizontal sections of the brain taken from the rat No. 3163 (sham), No. 3155 (hippocampal lesion) and No. 3164 (hippocampal lesion) 3 or 4 weeks after lesion surgery and stained with fast cresyl violt. The level of the sections are, at 3.5, 4.0, 5.0 and 7.0 mm ventral to the bregma (according to the atlas of Paxinos and Watson, 1986)

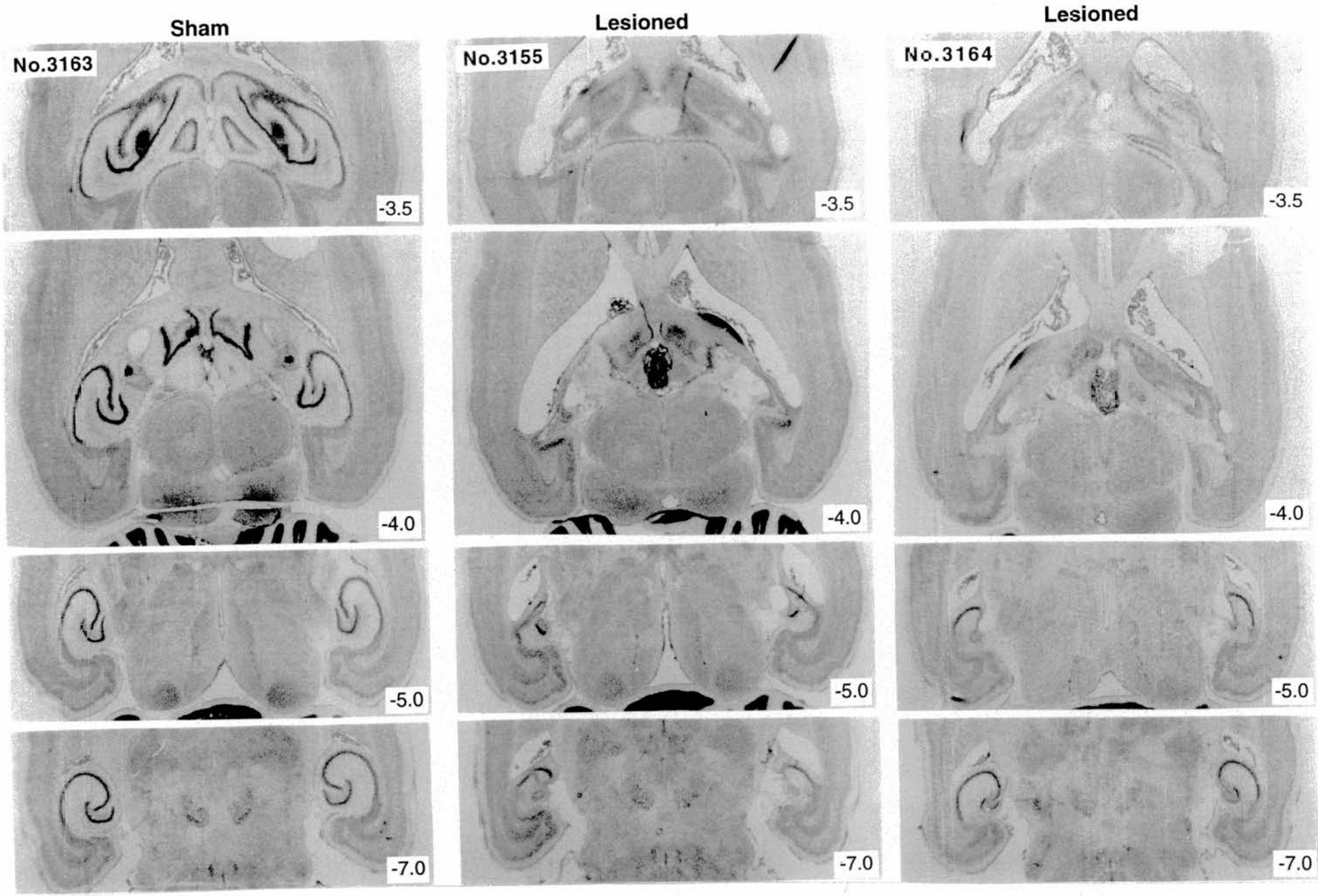

Sham
Lesioned
Lesioned
No.3163
No.3155
No.3164
-3.5
-3.5
-3.5
-4.0
-4.0
-4.0
-5.0
-5.0
-5.0
-7.0
-7.0
-7.0

Fig. 5-1

Table 5-1 Histological evaluation of hippocampal lesion (3 or 4 weeks after injection of ibotenic acid)

Rat No.3152

	LEFT				RIGHT			
horizontallevel(Bregma=0)	DG	CA3	CA1	Sub	DG	CA3	CA1	Sub
- 3 ~ - 3.5 mm	++++	++++	++++	-	+++	++++	++++	-
- 3.5 ~ - 4 mm dorsal	+++	++++	+++		+++	++++	++	
- 3.5 ~ - 4 mm ventral	++++	++++	++++	-	++++	++++	++++	-
- 4 ~ - 6 mm	++++	++	++	-	++++	++++	++++	-
- 6 ~ - 8 mm	+++	++	++	-	++++	++	+++	-

Rat No.3154

horizontallevel(Bregma=0)	DG	CA3	CA1	Sub	DG	CA3	CA1	Sub
- 3 ~ - 3.5 mm	+++	+++	+++	-	++++	++++	+++	-
- 3.5 ~ - 4 mm dorsal	+++	++++	++++		+++	++++	++++	
- 3.5 ~ - 4 mm ventral	++	++++	++++	-	++++	++++	+++	-
- 4 ~ - 6 mm	++	+++	+++	-	+++	+++	++++	-
- 6 ~ - 8 mm	++++	++	++	-	+++	++	++++	-

Rat No.3155

horizontallevel(Bregma=0)	DG	CA3	CA1	Sub	DG	CA3	CA1	Sub
- 3 ~ - 3.5 mm	++++	++++	++++	++	++++	++	+++	s
- 3.5 ~ - 4 mm dorsal	+++	++++	++++		+++	++++	++++	
- 3.5 ~ - 4 mm ventral	++++	++++	++++	-	++++	++++	++++	-
- 4 ~ - 6 mm	++	+++	+++	-	++	+++	+++	-
- 6 ~ - 8 mm	++++	++	++	++	+++	++	++	-

Rat No.3161

horizontallevel(Bregma=0)	DG	CA3	CA1	Sub	DG	CA3	CA1	Sub
- 3 ~ - 3.5 mm	++++	+++	++++	-	+++	++++	++++	-
- 3.5 ~ - 4 mm dorsal	+++	++++	++++		+++	++++	++++	
- 3.5 ~ - 4 mm ventral	+++	+++	++	-	+++	++++	++++	-
- 4 ~ - 6 mm	++++	++	++	-	++	++++	++++	-
- 6 ~ - 8 mm	++++	++	s	-	+++	++	++	-

Rat No.3164

horizontallevel(Bregma=0)	DG	CA3	CA1	Sub	DG	CA3	CA1	Sub
- 3 ~ - 3.5 mm	++++	++++	++++	s	++++	++++	++++	s
- 3.5 ~ - 4 mm dorsal	+++	++++	++++		+++	++++	++++	
- 3.5 ~ - 4 mm ventral	++++	++++	++++	s	++++	++++	++++	s
- 4 ~ - 6 mm	++++	+++	++	-	++++	++++	+++	-
- 6 ~ - 8 mm	++	s	s	-	++	s	s	-

Rat No.3166

horizontallevel(Bregma=0)	DG	CA3	CA1	Sub	DG	CA3	CA1	Sub
- 3 ~ - 3.5 mm	++++	++++	++++	++	++++	++++	++++	++
- 3.5 ~ - 4 mm dorsal	+++	++++	++++		+++	++++	++++	
- 3.5 ~ - 4 mm ventral	++++	++++	++++	s	++++	++++	++++	s
- 4 ~ - 6 mm	++++	++++	++++	-	++++	++++	++++	-
- 6 ~ - 8 mm	++++	++	++	-	++++	++	++	-

++++ : complete lesion (100 ~ 95 % removal of the neurons)
+++ : almost complete lesion (95 ~ 80 % removal of the neurons)
++ : partial lesion (80 ~ 20 % removal of the neurons)
s : slight damage (20 ~ 5 % removal of the neurons)
- : intact (5 ~ 0 % removal of the neurons)

5.3.2 Body weight

In order to avoid having a significant difference in body weight between the sham group and the HL group during water maze training, assignment of animals was designed as follow. In the assignment of 12 animals into two groups before surgery, the heaviest 4 animals were assigned to HL group and the lightest 4 animals were assigned to sham group. The mean body weight of the two groups was significantly different from each other before surgery ($p < 0.05$ and the results of statistical calculations are shown in Table 5-2 and 5-3). As the mean body weight of HL group was slightly decreased 1 week following the last surgery (Fig. 5-2), there was no statistical difference in body weight between two groups just before the acquisition training (2 weeks following surgery).

5.3.3 Place navigation training (Day 1 ~ Day 4)

None of the animals had any difficulty or disturbance in their motor ability to perform place navigation task, e.g. swimming around the pool or climbing onto the platform.

The sham and HL groups showed a progressive decline in escape latency across trials (Fig. 5-3) and with days (Fig. 5-4). Although the progress in acquisition in the HL group was a little slower than that of sham group (see performance of HL group on Day 2 in Fig. 5-4), the ANOVA of mean escape latency for trial (Table 5-4) or for day (Table 5-5) revealed no significant difference between groups, in addition, the latency of both groups reached a similar asymptotic level by the end of training (Fig. 5-3, Fig. 5-4).

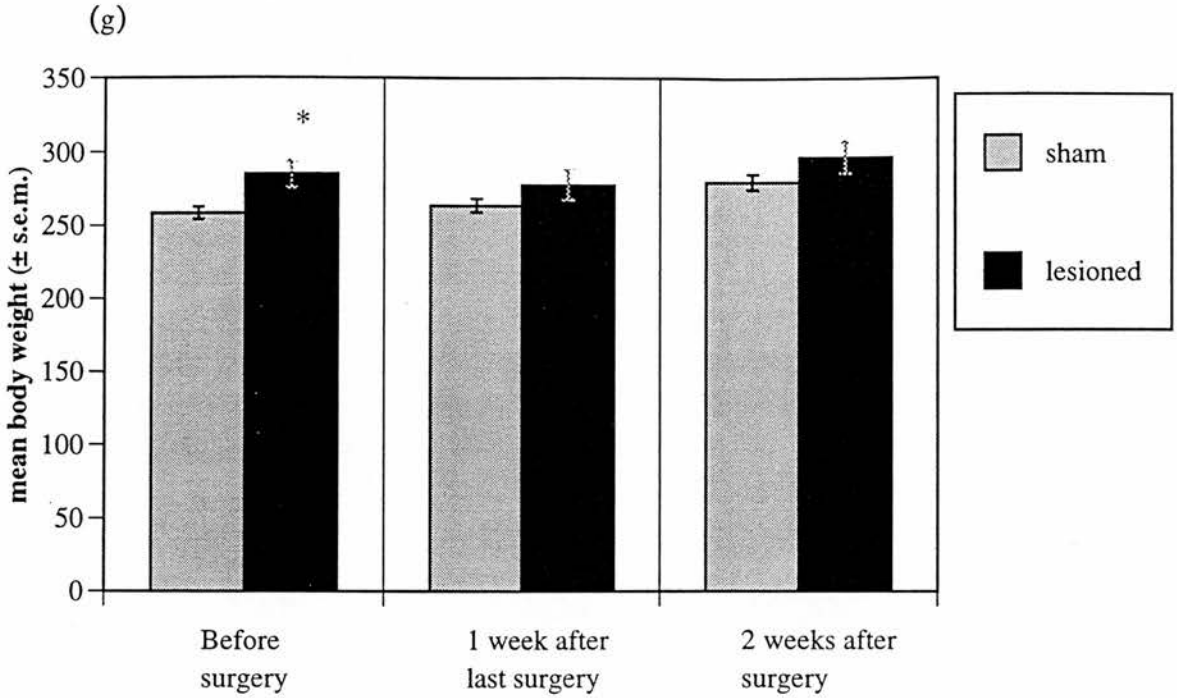


Fig. 5-2 Change in body weight of animals

Table 5-2 ANOVA of body weight

Group : 2 groups,12 animals (sham, hippocampal lesioned) **Day** : before ,1w after, 2w after surgery

source of variation	df	sum of squares	mean suqare	F	p
Group	1	3287.111	3287.111	3.107	0.1085
Error	10	10581.222	1058.122		
Day	2	2258.000	1129.000	59.982	0.0000
Group × Day	2	248.222	124.111	6.594	0.0063
Error	20	376.444	18.822		

Table 5-3 T-Test for body weight

Sham group vs **Hippocampal lesioned group**

Day of measurement	Before surgery	1 week after surgery	2 weeks after surgery
t value	2.6167	1.2944	1.4022
degree of freedom	10	10	10
two-tailed P value	0.0257 *	0.2246	0.1911

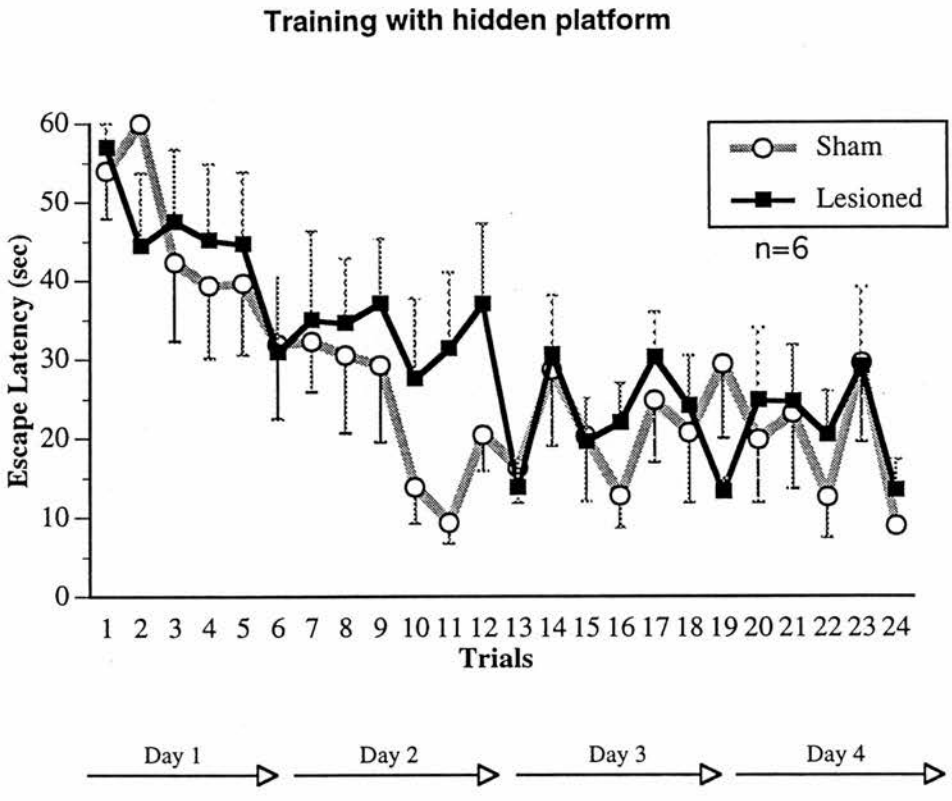


Fig. 5-3 Mean (+ or - s.e.m.) escape latencies for each trials.

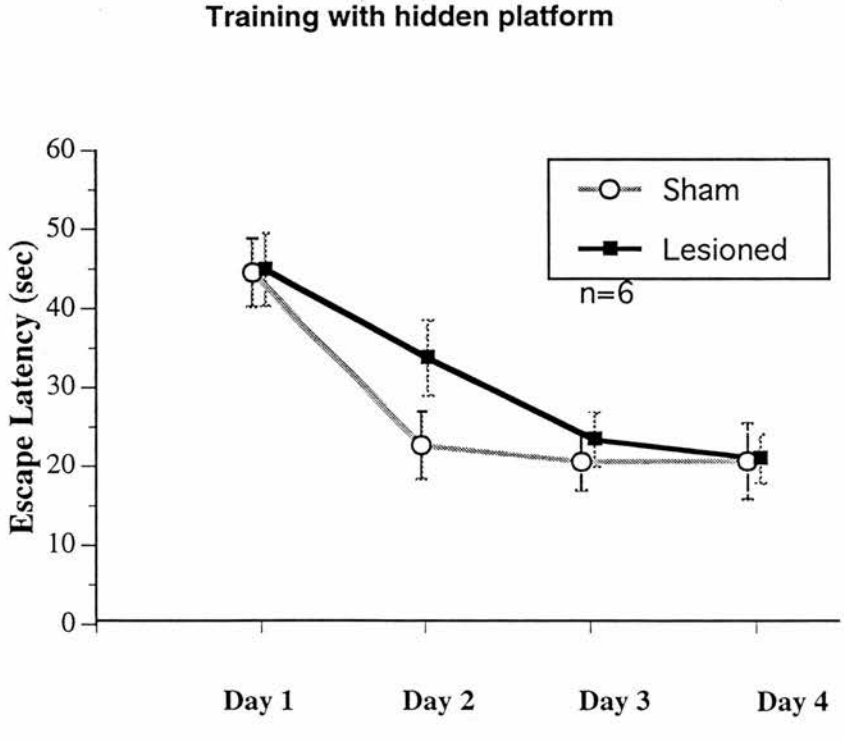


Fig. 5-4 Mean (± s.e.m.) escape latencies for each training day

Table 5-4 Analysis of performance in place navigation training

ANOVA of the mean escape latencies for **each trial**

Group: 2 groups (sham group, hippocampal lesion group) **Trial**: 24 trials

source of variation	degree of freedom	sum of squares	mean suqare	F	p
Group	1	957.396	957.396	0.849	0.3784
Error	10	11271.857	1127.186		
Trial	23	37279.732	1620.858	4.955	0.0000
Group × Trial	23	4641.048	201.785	0.617	0.9152
Error	230	75230.082	327.087		

Table 5-5 Analysis of performance in place navigation training

ANOVA of the mean escape latencies for **each day**

Group: 2 groups (sham group, hippocampal lesion group) **Day**: 4 days

source of variation	degree of freedom	sum of squares	mean suqare	F	p
Group	1	159.505	159.505	0.849	0.3785
Error	10	1878.777	187.878		
Day	3	4389.857	1463.286	19.361	0.0000
Group × Day	3	234.697	78.232	1.035	0.3912
Error	30	2267.368	75.579		

The swimming speed of both groups during place navigation training is compared in Fig. 5-7 (This graph also shows the swimming speed on trials on Day 5 which will be commented on in later sections). An ANOVA of swimming speed (Table 5-8) revealed a significant group effect ($p < 0.01$) and significant reduction of speed across days (Day effect in Table 5-8 $p < 0.05$) but no significant group by day interaction. T-test comparing the mean swimming speed for 4 days training showed that a significantly higher speed of HL group than sham group ($p < 0.01$). If the swimming speed is compared day by day, t-test revealed a significant difference between groups on Day 1 ($p < 0.01$), Day 2 ($p < 0.01$) and Day 3 ($p < 0.05$) (see Fig.5-7 and Table 5-8). As the escape latency was not statistically different, the HL group swam a longer distance to find the platform on these days.

The swimming speed of HL group on Day 4 was not statistically different from that of sham group. However, a close observation of animal behaviour found a slight difference in their performance. A sham animal swam directly towards the platform on one or two of the 6 trials on Day 4 (total 10 trials out of 36). In other trials they swam towards the platform with a zig zag pattern or swam straight to the vicinity of the platform and made a small search to find it. Examples of animal performance during place navigation training is shown in Fig. 5-8. This behaviour may indicate that they had to rely on both an inaccurate spatial memory of platform location and a random searching skill. Their strategy on Day 4 was not developed in comparison with that on Day 3. The HL animals swam directly to the platform in only 6 trials out of total 36 trials on Day 4. They mostly used a circular search strategy in which two dimensional spatial memory was not necessary. This difference of performance between sham group and HL group in the final stage of spatial learning was substantially revealed by the performance in the following transfer test.

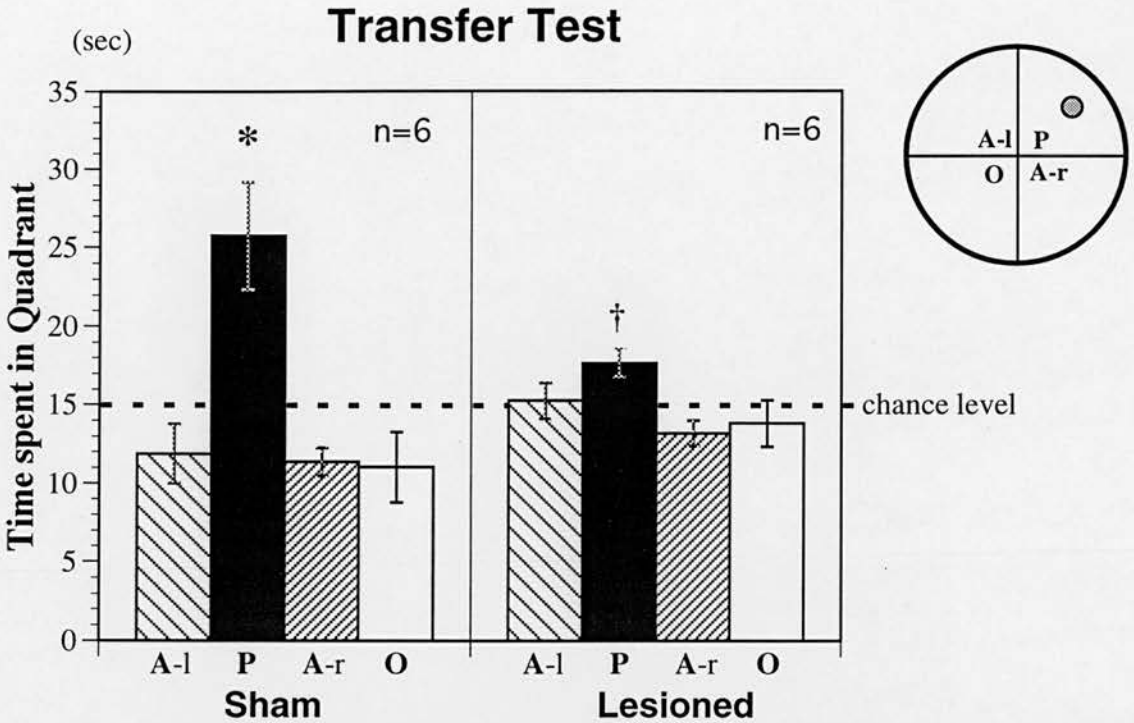


Fig. 5-5 Mean (±s.e.m.) time spent in each Quadrant during 60 sec transfer test. (P: quadrant where the escape platformwas located during trainig, A-l, A-r : quadrant ajacent to P, O: quadrant opposit to P)

* : $p < 0.05$ P vs O quadrant (Tukey multiple comparison test following ANOVA of time spent in each quadrant. see Table 5-6)

† : $p < 0.05$ hippocampal lesion vs sham (T-test of time spent in P quadrant $t = 2.283$)

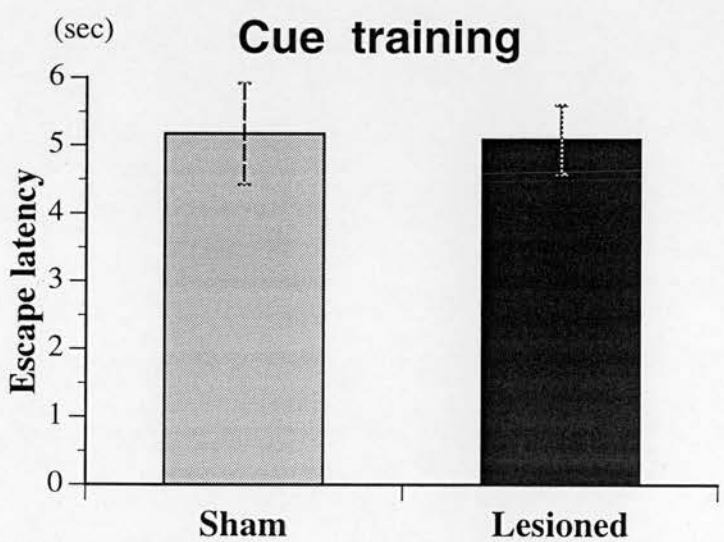


Fig. 5-6 Mean (± s.e.m.) escape latency in the cue trainig Unpaired t test : t=0.0941 The tow tailed P value is 0.9269 The difference between twp groups is not significant

Table 5-6 Performance of **Sham** animals in **transfer test**

Repeated measures **ANOVA** of time spent in each of **4** quadrants

source of variation	degree of freedom	sum of squares	mean suqare	F	p
Quadrants	(3)	928.77	309.59	19.157	0.0031**
Subjects	5	0.0071	0.0014		
Error	(15)	637.81	42.521		

Repeated measures **ANOVA** of time spent in each of **3** quadrants

source of variation	degree of freedom	sum of squares	mean suqare	F	p
Quadrants	2	821.95	410.98	6.7809	0.0138*
Subjects	5	7.7244	1.5449		
Error	10	606.08	60.608		

Tukey Multiple comparison test following the above ANOVA

Comparison	P value (4 quadrants)	P value (3 quadrants)
P quadrant vs **A-l**	$p < 0.05$ *	$p < 0.05$ *
P quadrant vs **O**	$p < 0.01$ **	$p < 0.05$ *
P quadrant vs **Ar**	$p < 0.01$ **	
o quadrant vs **A-l**	$p > 0.05$	$p > 0.05$
	$p > 0.05$	
A-l quadrant vs **A-r**	$p > 0.05$	

Table 5-7 Performance of **Lesioned** animals in **transfer test**

Repeated measures **ANOVA** of time spent in each **4** quadrant

source of variation	degree of freedom	sum of squares	mean suqare	F	p
Quadrants	(3)	71.828	23.943	2.4270	0.1059
Subjects	5	0.0071	0.0014		
Error	(15)	147.97	9.865		

Repeated measures **ANOVA** of time spent in each **3** quadrant

source of variation	degree of freedom	sum of squares	mean suqare	F	p
Quadrants	2	45.788	22.894	1.8740	0.2036
Subjects	5	6.4561	1.2912		
Error	10	122.17	12.217		

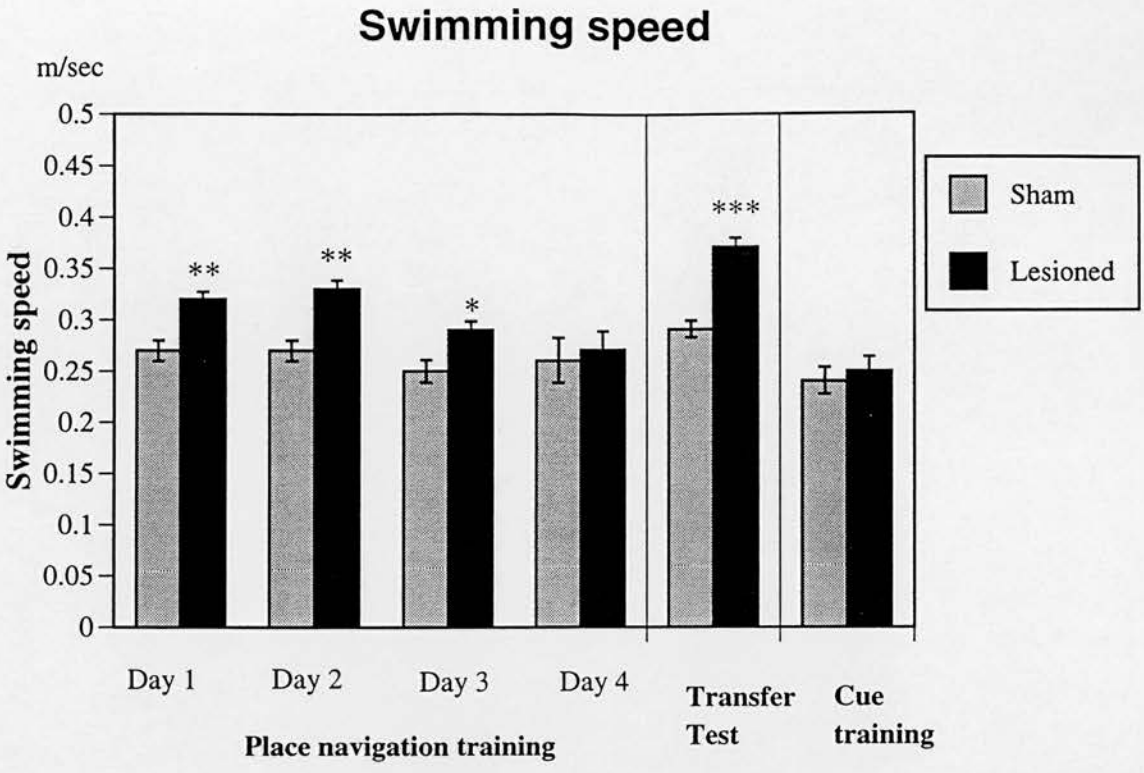


Fig. 5-7 Mean (± s.e.m.) swimming speed during each training or testing.

*** : $p < 0.0001$ vs Sham
** : $p < 0.01$ vs Sham
* : $p < 0.01$ vs Sham (t-test)

Table 5-8 Analysis of swimming speed

ANOVA of the swimming speed dring place navigation training

source of variation	degree of freedom	sum of squares	mean suqare	F	p
Group	1	0.019	0.019	15.738	0.0027**
Error	10	0.012	0.001		
Day	3	0.012	0.004	4.03	0.0160*
Group × Day	3	0.003	0.001	0.97	0.4198
Error	30	0.029	0.001		

T-test Sham vs Hippocampal lesion

Day1 ($p < 0.01$) ; Day 2 ($p < 0.01$) ; Day 3 ($p < 0.05$) ; Day 4 ($p = 0.59$)

Unpaired t-test of swimming speed in **Transfer test**

Mean difference = 0.0783

t = 6.4559 with 10 degree of freedom

The two-tailed P < 0.0001***

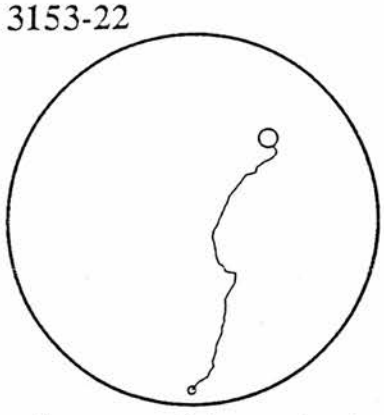
Unpaired t-test of swimming speed in **Cue training**

Mean difference = 0.0083

t = 0.4235 with 10 degree of freedom

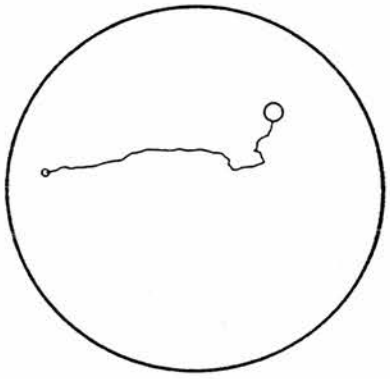
The two-tailed P = 0.6809

3153-22



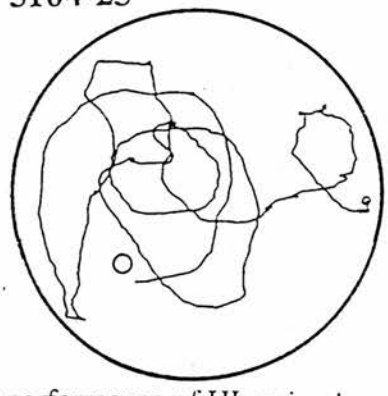
performance of sham animal
on Day 4

3156-24



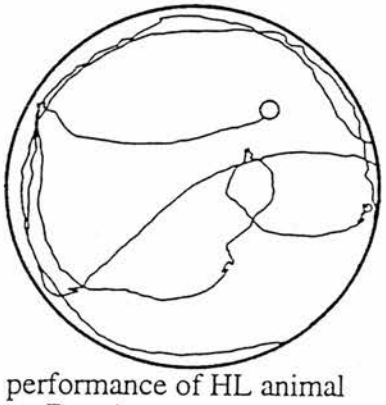
performance of sham animal
on Day 4

3164-23



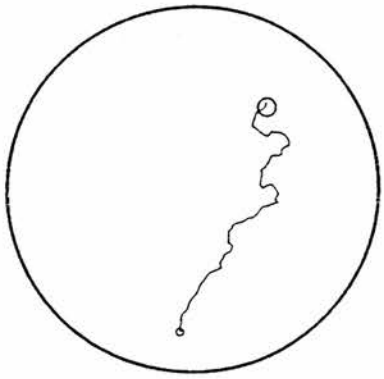
performance of HL animal
on Day 4

3154-23



performance of HL animal
on Day 4

The example that took long
time to find platform because
of the innacurate memory

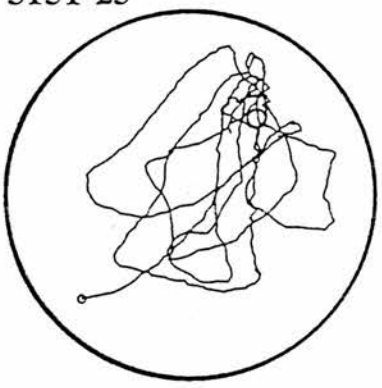


zig zag searching pattern

Fig. 5-8 Examples of performance in the place navigation training

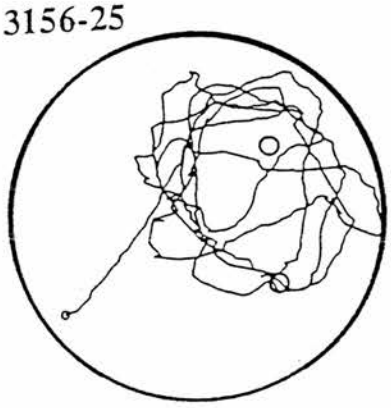
large circle: pool
medium circle: position of hidden platform
small circle: start point

3151-25



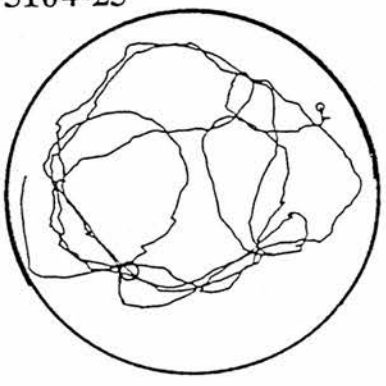
performance of sham animal

3156-25



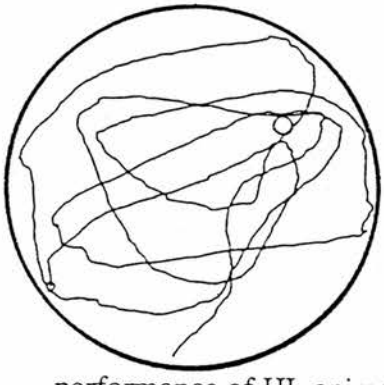
performance of sham animal
proper bias to NE quadrant but
did not reach exact point

3164-25



performance of HL animal

3153-25



performance of HL animal

no swimming bias
but reached the exact point
immediately

Fig. 5-9 Examples of performance in the transfer test

large circle: pool
medium circle: position where the platform had been located during training
small circle: start point

Although all rats usually stayed on the platform for 30 sec after climbing onto it at the end of each trial, some rats dived into the water or jumped up and fell in water before 30 sec had elapsed. In that case, the rats were brought back onto the platform by the experimenter and made to stay on it for 30 sec to ensure they had enough time to see the extra maze cue from the platform. In the training of sham group, such incidents took place in 21 trials out of a total of 144 trials (total 6 trials x 4 days x 6 animals). In the training of HL group, such incidents took place in 12 trials out of 144 trials. Fisher's exact test revealed that there is not a significant association between number of incidents and groups ($p = 0.138$).

5.3.4 Transfer Test

Examples of performance of both groups of animals in the transfer test is shown in Fig. 5-9

The mean time spent in each quadrant of the pool during 60 sec test swimming is shown in Fig. 5-5. While the sham rats stayed for longer time in the 'P quadrant' in which the platform had been located during training trials than in any other quadrants, HL rats did not show such swimming bias during 60 sec test period. In order to assess the rat's memory of the platform location, the mean time spent in the 'P quadrant' was compared to the time spent in other quadrants. As total time spent in all 4 quadrants was fixed at 60 sec (i.e. the data of all 4 quadrants are not completely independent each other), mean time spent in 3 quadrants out of 4 was analysed.

Repeated measures (one-way within subjects) ANOVA of sham group (Table 5-6) revealed a significant difference between quadrants [$F = 6.78$, $p < 0.05$]. Further analysis using Tukey multiple comparison test showed that the time spent in

the P quadrant is significantly longer than the time spent in the opposite side quadrant (O) or one of the adjacent quadrant (A-l) ($p < 0.05$, Table 5-6). On the other hand, ANOVA of HL group did not show any significant effect of quadrant ($p = 0.2036$, Table 5-7).

Since the result of 3 quadrants analysis is variable according to the combination of selected quadrants, analyses of 4 quadrants are also shown in Tables 5-6 and 5-7 for reference. The results of 4 quadrants analysis are shown to be basically similar to those of 3 quadrants analysis.

Therefore, there is a striking contrast between the performance of the sham group and that of the HL group. While sham group remembered the position of the platform and searched significantly longer time in the P quadrant, HL group did not show a swimming pattern to search in a particular quadrant.

In addition to the above within-subjects analysis, a significant difference of the performance between sham group and HL group in the transfer test is also revealed by the comparison between groups. T-test of the mean time spent in P quadrant revealed that sham group stayed for significantly longer time (25.8 ± 3.4 sec) in P quadrant than HL group (17.7 ± 0.9 sec) ($t = 2.283$ $P < 0.05$ Fig. 5-5). T-test of swimming speed revealed that HL group significantly swam faster than sham group ($p < 0.0001$) (Table 5-8).

5.3.5 Cue navigation training (Day 5)

The mean escape latency in the cue training is shown in Fig. 5-6. T-test revealed no significant difference between sham and HL group. The mean swimming speed of both group is not significantly different each other (Fig. 5-7, Table 5-8).

5.3.6 Environmental uniformity

If there were any intra- or extra-maze objects that guided animals to or drove animals away from a particular location in the pool, they could inhibit acquisition of spatial location. In order to check the uniformity of the maze environment, the performance of the rats trained with the platform in different locations was compared.

Three of sham animals and three of HL animals were trained with the platform located in the NE quadrant during place navigation training (NE group). The other three sham animals and three HL animals were trained with the platform located in the SW quadrant (SW group).

The escape latency in the place navigation training and cue navigation training is compared in Fig.5-10 (A). An ANOVA of escape latency in place navigation training shows no significant group effect [$F(1, 10) = 0.077$ $p = 0.78$], a significant day effect [$F(3,30) = 20.43$ $p < 0.0001$] and no significant interaction between group and day [$F(3,30) = 0.78$ $p = 0.510$]. T-test of escape latency in cue navigation training shows the difference between two groups is not significant [$t = 0.227$ $p = 0.82$].

Swimming speed was also compared (Fig.5-10 (B)). An ANOVA of swimming speed during place navigation training revealed no significant group effect [$F(1, 10) = 1.47$ $p = 0.25$], a significant day effect [$F(3,30) = 4.357$ $p < 0.05$] and no significant group by day interaction [$F(3,30) = 1.86$ $p = 0.16$]. T-tests of swimming speed in the transfer test and in the cue navigation training did not indicate significant difference between groups ($t = 0.946$ $p = 0.366$ or $t=1.163$ $p = 0.27$ respectively). These results do not suggest the existence of environmental bias.

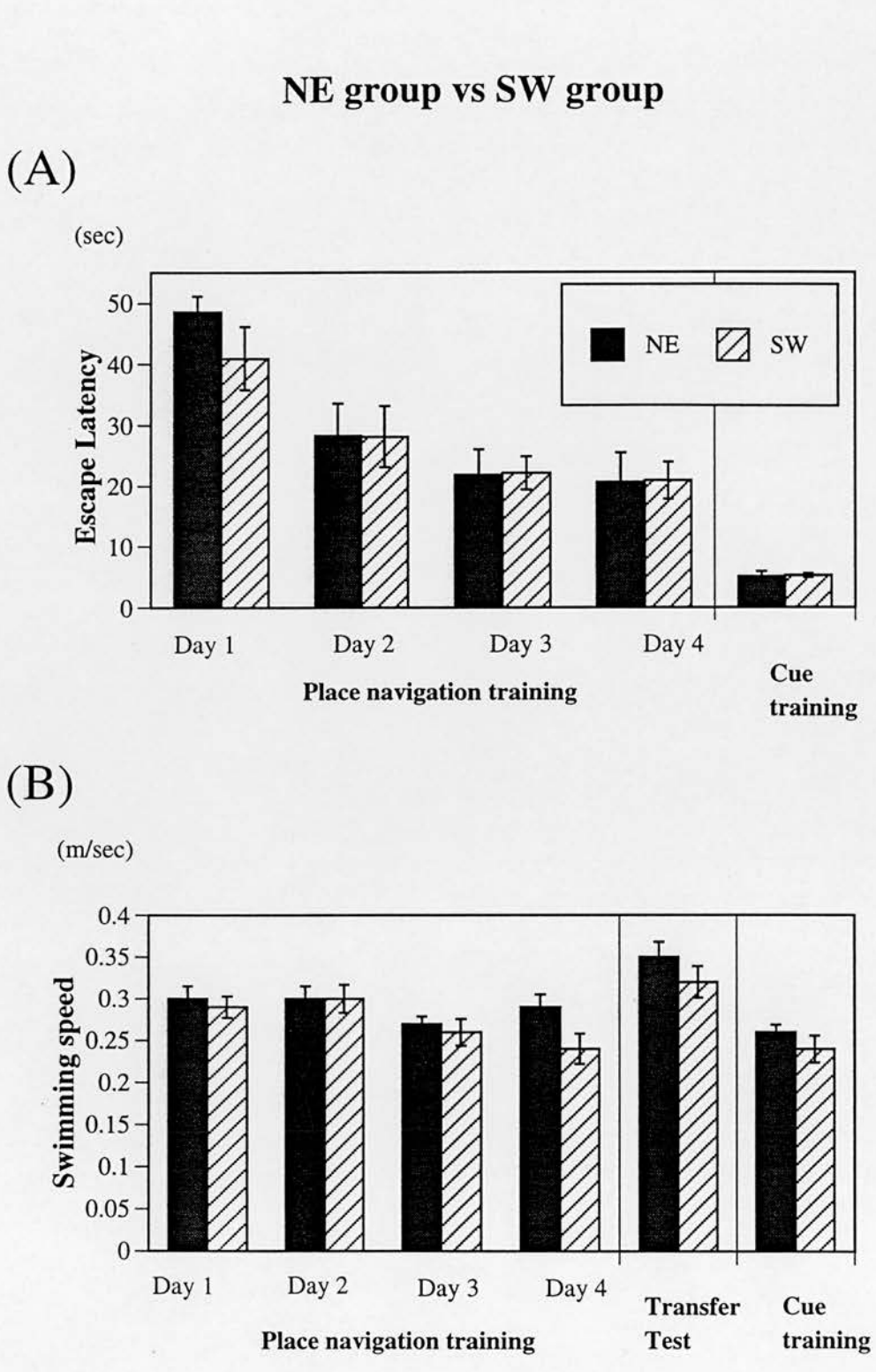


Fig. 5-10 Comparison of performance between NE group and SW group.

5.4 Discussion

The results of the transfer test demonstrated that the hippocampal lesioned animals did not acquire the memory of the platform after 4 days place navigation training while sham animals acquired. This impairment of learning in HL animals is considered to be due to the lack of ability for memory acquisition, rather than to a delayed acquisition process because the escape latency of both groups reached an asymptote on Day 4 and further improvement or progress could hardly be expected.

The learning deficit of HL animals in the present experiment revealed by the transfer test is consistent with the deficit of hippocampal lesion animals reported by Morris et al. (1982) and AP5 treated animals reported by Morris (1989), and Davis et al. (1992). All these results suggested that the place navigation learning in the water maze task is sensitive to the damage in hippocampal function.

However, the above cited experiments showed learning deficit of hippocampal lesion or AP5 animals not only by the poor swimming bias in the transfer test but also by,

(1) a significantly longer escape latency in the early stage of place navigation training

(2) a higher asymptote level of escape latency in the last stage of training.

In the present results, on the other hand,

(1') HL group showed a little but not significantly longer escape latency than sham group.

(2') The escape latency of HL group and sham group reached a similar asymptotic level by the end of training.

The present experiment set the time limit for each trial at 60 sec while in the other reported experiments it was set at 120 sec. In the 120 sec experiment, the performance of poor learners increases the mean escape latency much greater than in

the 60 sec experiment. In order to check the effect of the shorter maximum time on the results, the raw data was transformed by changing all escape latencies of 60 sec to 120 sec and recalculated. For example, the mean (± s.e.m.) escape latency of sham group on Day 2: 22.61(±4.31) sec was changed to 27.61(±7.35) sec while that of HL group: 33.73(±4.73) sec was changed to 53.73(±12.37) sec. However, ANOVA of transformed escape latency still showed no significant group effect [$F(1, 10) = 0.78$ $p = 0.397$] nor a significant group by day interaction [$F(3, 30) = 1.78$ $p = 0.172$]. (Group x trial ANOVA also showed no group effect). Thus, the shorter maximum latency does not seem to mask the poor performance of HL group.

Although the analysis of escape latency did not detect the difference between groups, the analysis of swimming speed revealed a significant difference between sham and HL group. The HL group swam significantly faster than sham group on Days 1, 2 and 3. It means that the HL group found the platform after a search with longer distance than the sham group. Therefore, performance of HL animals in the present experiment was poorer than that of sham group at an early stage of training. From a standpoint of escape efficiency, the difference between (1) and (1') may not be fundamental. However, on Day 4 (the last day of training) the performance of sham and HL animals reached the same level and there is no measurable difference even using the above measure (2'). On the other hand, a clear contrast was revealed between the performance of sham and HL animals in the transfer test which showed that the sham animals obviously acquired some information about the platform position. Therefore it is explained that the spatial memory of sham animals was not precise enough to find the platform quicker than the HL animals searching the pool randomly. In addition, it seems that the sham animals did not feel the necessity to improve their performance further on Day 4. While the control animals in the other published study are reported to take a direct path to the platform and the mean escape latency is about 10 sec on the last day of training, the sham rats in the present

experiment did not always take direct paths and took about 20 sec to find the platform in the pool of the same size. That 20 sec latency is also found to be too long for direct escape behaviour by the fact that the escape latency was only about 5 second in the cue navigation training in Experiment 3. This evidence shows that the result (2') is caused by the poor performance of sham animals rather than the exceptionally better performance of HL animals.

The possible cause for the poor performance of the sham group is the modification of the water temperature and the maximum swimming time for each trial in the Experiment 3. These modification were made in the attempt to reduce stress on the first day on which sometimes makes rats refuse to take normal and effective escape behaviour (e.g. swim round and round keeping close to the side wall and never try to seek escape in the central area). However, the reduction in stress level in training is also considered to have an adverse effect on the motivation to make a precise quick escape. As reported by McNaughtom and Morris (1987b) or McNamara and Skelton (1991), treatment with anxiolytic drugs or an increase of water temperature impairs spatial learning in the water maze probably by suppressing motivation to escape from water. In order to cope with this problem, the modification was mainly targeted only to the procedure on the first day of place navigation training in the Experiment 3. The water temperature was raised only on Day 1 (2°C higher than the standard experiments). The rescue of rats after 60 sec limit was mainly carried out on Day 1 because the rat escaped within 60 sec on most of the trials of following days (e.g. sham rats failed to escape within 60 sec on only 3 trials out of 36 on Day 2). It is amazing this slight modification on Day 1 had effect on the final performance.

Although the spatial memory acquired by sham group may not be precise enough to show clearly more effective escape than that of HL group in the final day of training, the acquisition of that memory itself and its dependence on the

hippocampal function was demonstrated by the clear contrast in the acquisition of swimming bias in the transfer test between sham group and HL group.

Therefore, this learning model is still considered to be useful to evaluate drug's effect on the animal's ability of spatial memory acquisition which is dependent on hippocampal function. We should keep in mind that the higher efficiency of finding the escape platform (shorter escape latency) does not necessarily represent the acquisition of spatial memory of platform location in this experimental condition. The spatial memory is only represented by the searching bias in the transfer test.

Chapter 6
Experiment 4: Water Maze study (1)
Experiment 5: Water Maze study (2)
Experiment 6: In vivo LTP study (2)

This chapter discusses two water maze experiments and a supplementary LTP experiment. Experiment 4 is an investigation of the dose and time dependence of the effect of FR115427 and MK-801 on spatial learning. Experiment 5 involves a modified pre-training procedure and changed water temperature. Experiment 6 was conducted in the same animals that were tested in the water maze to examine the effect of repetitive injection of FR115427.

6.1 Experiment 4

6.1.1 Introduction

The preceding investigations of the effect of FR115427 on open field activity and LTP showed that the MK-801 like motor syndrome and inhibitory effect on LTP were exhibited according to different time courses. The former was a quick onset effect and the latter a late onset effect.

The main purpose of the studies of this chapter are to investigate: (1) whether FR115427 has any effect on spatial learning; and (2) establish whether it is the early

onset type or the late onset type because the time course is expected to be a key to understanding the mechanism of any learning effect of the drug. Accordingly, some groups were given a short interval (20 min) between drug administration and training in the water maze, others a long interval (90 min), both being carried out in parallel and the results compared. In addition to saline control groups, MK-801 groups were also tested as a reference to compare the results with that of the other reported water maze experiments using MK-801 discussed in Chapter 1.

6.1.2 Methods

(a) Assignment of drug dosage

MK-801 was tested at two doses 0.1 and 0.032 mg/kg. The top dose, 0.1 mg/kg, is the maximum dose at which no significant ataxic effect was observed in Experiment 1 (Chapter 3) and significant impairments of learning in the water maze have previously been obtained (Chapter 1 Table 1-1). The second dose is lower than that at which impairments of learning was reported. FR115427 was tested at 4 doses 10, 3.2, 1.0 and 0.32 mg/kg. The 10 mg/kg is the maximum dose at which significant ataxic effect was not detected in the Experiment 1. At this dose, FR115427 blocked LTP in Experiment 3. The lower doses 3.2, 1.0 and 0.32 mg/kg are approximately equivalent to 0.1, or 0.032 mg/kg of MK-801 as an NMDA receptor antagonist. Thus, there were 7 different drug groups including the saline group. Using both the short and long intervals between injection and training making a total of 14 different groups in the experiment.

(b) Assignment of animals

This experiment was run in replicates each consisting of 7 or 8 animals. Each animal was randomly assigned to one of 14 different experimental groups as shown in Table 6-1. As 6 animals were finally allocated to each group, a total of 84 animals were used in Experiment 4.

(c) The procedure for training and testing

Each replicate in Experiment 4 exactly followed the schedule shown in Fig.2-3 (Chapter 2) All rats received handling and pretraining trials (2 trials)in the first week. In the 2nd week, animals had place navigation training for 4 days (Day 1 ~ Day 4, 6 trials per day). The position of the submerged platform was fixed at one location for each animal. As shown in Table 6-1, 38 out of 84 animals were trained to locate the platform in the NE quadrant and the rest of the animals were trained to locate the platform in SW quadrant. About 24 hours after the last training (on Day 5), the animals received a single transfer test followed by cue navigation training (6 trials). Drugs were administered 20 min or 90 min before the commencement of training or test of each day. The water temperature was kept at 25.0 ± 0.5°C except on Day 1 on which the temperature was kept at 27.0 ± 0.5 °C.

(d) Trials that rats refused to escape onto the platform

Some animals, especially some in the high drug dose groups, were so anxious or nervous that they showed no intention of escaping onto the submerged platform and stayed close to the side walls of the pool during training even on Day 4. In the worse case, the rats did not stay voluntarily on the platform when the experimenter placed them on the platform after 60 sec time limit of each trial. They jumped up to the side wall or dived into the water and swam away from the platform. In such cases, the rats were picked up again and returned to the platform and, if necessary, restrained there

Table 6-1 Assignment of Animals in each replicate of Experiment 4

The same procedure of training and testing was repeated 11 times.
The number represents the assignment of animals to one of 14 groups.

No.of replicate	Group														position of hidden platform
	20 min							90 min							
	Saline	MK 0.032	MK 0.1	FR 0.32	FR 1.0	FR 3.2	FR 10	Saline	MK 0.032	MK 0.1	FR 0.32	FR 1.0	FR 3.2	FR 10	
1	1		1			2	1		1		1	1			SW
2		1		1	1					1			1	2	SW
3		1		1		1	1	2		1		1			NE
4	1		1		1	1			1		1		1	1	NE
5	1	1			1	1				2	1			1	SW
6	1	1	1				1	1	1			1	1		SW
7			1	1	1		1	1	1				1		NE
8	1	1		1						1	1	1	1	1	NE
9	1		1		1		1	1	1		1		1		SW
10		1		1	1		1	1		1		1			SW
11			1	1		1			1		1	1		1	NE
Total No of animals	6	6	6	6	6	6	6	6	6	6	6	6	6	6	

by hand for 30 sec to secure sufficient time for them to see the spatial relationship between the platform and the extra maze cues.

Training trials on which an animal did not stay voluntarily on the platform for 30 sec were recorded as 'refused trials'. The rats which showed normal escape behaviour also sometimes dived into the water before 30 sec had elapsed and looked for another possible escape. Such trials were also counted as 'refused trials' although they actually did not refuse to escape to the platform. Fisher's exact test was applied to compare the numbers of refused trial between two groups. As each group (N = 6) had 144 trials (6 trials x 4 days x N), the numbers of each incident were expressed as a proportion of the total 288 trials (144 x 2 groups) in the 2 x 2 test table.

6.1.3 Results of Experiment 4

20 min interval groups

(a) Place navigation training

Fig. 6-1 (page 150) shows the mean (± s.e.m.) escape latencies for each group over 4 days training. In order to avoid making the figure complicated, the results of MK-801 groups and FR115427 groups are shown in separate graphs. The results of the saline group is shown in both graphs of the figure as a reference to compare. All groups except FR 10 mg/kg group showed a progressive decline in escape latency across days. The performance of FR 10 mg/kg became worse on Day 2 and could not catch up with the level of the other groups by Day 4. One-way between and one-way within-subjects ANOVA of escape latencies revealed a significant main effect of group [$F(6,35) = 4.15$ $p < 0.01$] and day [$F(3,105) = 64.545$ $p < 0.001$]. The significant interaction term (group x day) [$F(18,105) = 2.01$ $p < 0.05$] allows analysis of

performance on each day. ANOVA of escape latency on Day 2 and on Day 3 revealed significant group effects [$F(6, 35) = 5.52$ $p < 0.001$, $F(6, 35) = 6.99$ $p<0.0001$ respectively]. The following Dunnett multiple comparison test revealed that only the performance of FR 10 mg/kg was significantly different from that of saline group on Day 2 ($p < 0.01$) and on Day 3 ($p < 0.01$). While the escape latency of MK-801 was slightly longer than that of the saline group throughout the 4 days training, the difference was not statistically significant.

The animals receiving FR 10 mg/kg showed a strong aversion to the water. They frequently vocalized and refused to stay on the submerged platform at the end of trials. As shown in Fig.6-6 (A) (page 155), the count of 'refused' trials reached a total of 61. Fisher's exact test (2x2) revealed that this number of incidents was significantly greater than the number of incidents observed in the saline group ($p < 0.0001$). Other groups did not show significantly higher incidents than the saline group.

No notable disturbance of swimming was observed in any group. Swimming speed during training is shown in Fig.6-5 (page 154). Although the swimming speed of MK 0.1 mg/kg group was slightly lower and the speed of FR 1 mg/kg group was slightly higher than other groups, one-way between and one-way within-subjects ANOVA on swimming speed during place navigation training showed no significant group differences [$F(6,35) = 1.73$ $p = 0.14$]. The main effect of day was significant [$F(3,105) = 12.14$ $p < 0.001$], and the interaction term (group x day) was not significant [$F(18,105) = 0.970$ $p = 0.50$]. This results reflects the tendency of all groups to decrease swimming speed in the latter phase of training (on Day 3 or Day 4) as they become more careful in their approach to the platform.

(b) Transfer test

Fig. 6-3 (A) (page 152) shows mean (+ s.e.m.) time spent in each quadrant during 60 sec transfer test. All groups, except the FR 10 mg/kg group showed a proper bias of swimming to the 'P quadrant' in which the escape platform had been located during training. As previously described in Chapter 5, repeated measures (one-way between subjects) ANOVA of time spent in 3 (P, Al and O) out of 4 quadrants was applied to the performance of each group to examine whether the above bias is statistically significant. The degrees of freedom of quadrant, subjects and residual error measure was 2, 5 and 10 respectively in each analysis. The ANOVA of all groups except FR 10 mg/kg group revealed a significant quadrant effect ($p < 0.001 \sim p < 0.05$ see Fig. 6-3 (A))(page 152). Tukey multiple comparison test confirmed that the time spent in P quadrant was significantly longer than that in the O quadrant or Al quadrant ($p < 0.001 \sim 0.05$) in all groups except FR 10 mg/kg group. The results of ANOVA in FR 10 mg/kg (20 min interval) group was that $F = 1.07$ $p = 0.38$. This group failed to acquire the memory of platform location after 4 days training.

The swimming speed of each group during transfer test is shown in Fig. 6-5(A) (page 154). ANOVA of swimming speed of 20 min groups did not detect any significant group differences [$F(6,35) = 0.86$ $p=0.53$].

(c) Cue navigation training

The mean (+ s.e.m.) escape latencies during 6 trials of cue navigation training are shown in Fig. 6-4 (A) (page 153). The escape latency of saline group was a little longer than the other groups because some of the saline animals still searched for the hidden platform during cue navigation trials. The difference between groups was not statistically significant according to the ANOVA (the main group effect ; [$F(6,35)=1.167$ $p=0.172$]). There were no 'refused' trials in this cue training.

Mean (± s.e.m.) swimming speed of each group during this training is given in the Fig.6-5(A) (page 154). Analysis of swimming speed did not indicate a significant group effect either [$F(6,35) = 0.76$ $p = 0.61$].

90 min interval groups

(d) Place navigation training

The mean (± s.e.m.) escape latencies are shown in Fig.6-2 (page 151). Although progressive decline of escape latency was observed in all groups, MK 0.1 mg/kg and FR 10 mg/kg group showed notably longer escape latencies. A one-way between and one-way within subjects ANOVA indicated a significant main effect of group [$F(6,35) = 2.38$ $p = 0.049$]. Because this significance was marginal, Tukey pairwise comparisons did not show any significant difference between any combination of groups. Only a Duncan's analysis detected significant differences ($p < 0.05$) between the saline group and the MK-801 0.1 mg/kg group, and between the saline and the FR 10 mg/kg group. The above ANOVA also detected a significant effect of day [$F(3,105) = 44.97$ $p < 0.0001$]. The interaction term (group x day) was not significant [$F(18,105) = 0.74$ $p = 0.76$] these effects reflected the uniform tendency of decreased latency by day in all the groups.

Some of the rats in MK 0.1 mg/kg and FR 10 mg/kg groups showed anxious behaviour like vocalization, swimming at the perimeter of the side walls and refusal to stay on the submerged platform. The incident of 'refused' trial observed in these groups was significantly higher than saline group (both $p < 0.0001$ Fisher's exact test) (Fig. 6-6 B) (page 155). However, the number of incidents in FR 10 mg/kg 90 min interval group was significantly smaller than that of FR 10 mg/kg 20 min interval group ($p < 0.05$). This smaller number of incidents in the 90 min group agreed with

the better performance of this group than the 20 min group in training trials. In contrast to the FR groups, the number of 'refused' trials was larger in the MK 0.1 mg/kg 90 min than that in the 20 min group. Fisher's exact test between MK 0.1 mg/kg 90 min and 20 min group revealed a significant difference ($p < 0.0001$).

A one-way between and within subjects ANOVA on swimming speed (Fig.6-5-B) (page 154) indicated a significant main effect of group [$F(6,35) = 2.41$ $p < 0.05$] and a significant main effect of day [$F(3,105) = 6.06$ $p < 0.001$]. The interaction between group and day was not significant [$F(18,105) = 0.87$ $p = 0.61$]. Subsequent post hoc Tukey or other comparison tests did not show any significant difference between saline group and other groups. The group effect was mainly due to the difference between the fast swimming FR 1 mg/kg group and slow swimming MK 0.1 mg/kg group.

(e) Transfer test

Fig. 6-3 (B) (page 152) summarises the performance of 7 groups during the transfer test. Although some groups showed a poor performance during place navigation training, all groups showed a proper searching bias to the platform quadrant. A significant quadrant effect was detected in all groups by repeated measures (one-way between subjects) ANOVAs of time spent in 3 (P, Al and O) quadrants ($p<0.05 \sim 0.001$ see Fig. 6-3 B) (page 152). Tukey post hoc test among quadrants confirmed that the time spent in Training quadrant was significantly longer than the time spent in O quadrant or Al quadrant ($p<0.05 \sim p < 0.001$).

Analysis of the swimming speed in the transfer test revealed a significant group effect [$F(6,35) = 3.558$ $p < 0.01$] (Fig. 6-5 B) (page 154). However, following Dunnett multiple comparison test did not detect any significant difference between saline group and other groups. Tukey multiple comparisons among all combinations of groups revealed the significant group effect was due to a difference between fast

swimming FR 1 mg/kg group and slow swimming MK-801 0.1 mg/kg or FR 10 mg/kg group ($p<0.05$).

(f) Cue navigation training

Analysis of escape latency during cue navigation training indicated no significant group effect [$F(6,35) = 0.63$ $p = 0.71$] (Fig.6-4 B) (page 153).

There were no significant difference in swimming speed among groups [$F(6,35)=1.416$ $p=0.236$] (see Fig.6-5B) and no 'refused' trials were observed during this cue training.

6.1.4 Discussion of Experiment 4

The main points in the results of Experiment 4 are:

(1) The FR115427 10 mg/kg group in the 20 min interval experiment failed to learn the platform location which should be expressed by a significant swimming bias to the platform quadrant in the transfer test.

(2) The FR115427 10 mg/kg group in the 90 min interval experiment did learn the platform location which was expressed as a significant swimming bias to the platform quadrant in the transfer test.

(3) The MK-801 0.1 mg/kg group learn the platform location in both the 20 min and the 90 min interval experiments.

(a) FR 10 mg/kg group in 20 min interval experiment.

FR10mg/kg, 20min group had place navigation training in a post-injection time interval at which LTP was partially inhibited but not completely blocked. Therefore, the severe learning deficit of this group could be a starting point of the argument that

the effect of non-competitive NMDA receptor antagonist on learning is nothing to do with the effect on LTP in the hippocampus. As the hippocampal lesion represents a complete loss of hippocampal plasticity, the learning deficit of the hippocampal lesion (HL) animals in Experiment 3 is compared to the performance of FR10mg/kg, 20min group in the first part of this discussion.

Although both the FR10mg/kg, 20min group and the HL group failed to acquire a significant swimming bias to P quadrant in the transfer test, some different points were observed between the performances of FR10mg/kg, 20min animals and HL animals. While the inefficiency of escape performance of HL animals (which completely lost hippocampal function) was only marginally detected by a slight increase in swimming path, the performance of FR10mg/kg, 20min was significantly and apparently deteriorated in comparison with that of the control group. The noticeable point of the performance of the FR10mg/kg, 20min group is that the escape latency was increased on Day 2 rather than decreased compared to on Day 1. These observation suggests that the drug not only negatively restrained the learning process but also positively deteriorated the learning performance. Furthermore, unlike in the HL group, there was a significant increase in the number of 'refuse' trial in the FR10mg/kg, 20min group. FR10mg/kg, 20min rats seemed to be strongly stressed and lost a regard of the submerged platform as a secure enough place to escape.

A loss of motivation to escape to the platform should be detected by a poor performance in training with the 'cue' platform which requires a similar behaviour but not the spatial memory. However, if the loss of motivation was caused by a rejection of the submerged platform itself as an safe refuge, the cue platform may not be a good control for test motivation because it protrudes above the water and enables the rats to get out from it completely. In addition, the cue navigation training is carried out after 4 days training by which time the rats possibly had learned to overcome their fear of the water. Thus the normal performance in the cue navigation training does not

guarantee the normal motivation level of animals during place navigation training. A training schedule started with cue raining and/or use of visible-submerged platform would seem to be required. In short, a difference of the regard for the escape platform between FR animals and HL animals is suggested by their escape behaviour.

The other difference between HL animals and FR animals was that HL animals received the transfer test after their performance in acquisition training reached an asymptote level whilst FR animals had still showed an improvement in their performance on the last day of training. There is a possibility that the learning of FR animals are just delayed but not blocked. Although a test with an extension of the training period seems to be an effective method to examine that point, there is a possibility that too much training can transform the nature of the learning in the water maze. Morris et al., (1990b) reported that the spatial learning deficit of the hippocampal lesion animals was eventually abolished after overtraining (rats received 76 trials training over 11 days) in the water maze. As discussed in Chapter 1, the animal with damage in the hippocampal system can identify and memorise a particular location in the pool after special training. (According to my idea, such learning can be achieved by utilizing the extramaze cues as a 'taxon' cue rather than by acquiring a 'spatial map'.) As it is difficult to define what extent of training is over training, and what level of performance is the asymptote performance, we have to decide whether the learning is "impaired" or "not impaired" in the defined period. Although it is difficult to discuss whether the learning is "blocked" or "delayed", there is a possibility that the learning in the FR10mg/kg, 20min animals was delayed.

To sum up, the impaired performance of FR10mg/kg, 20min group in the water maze seemed to be different from that of HL animals and such effect was observed in the equivalent condition in which FR115427 did not block LTP completely. The learning impairment in the FR10mg/kg, 20min group is possibly nothing to do with the inhibition of hippocampal plasticity.

(b) FR 10 mg/kg group in 90 min interval experiment.

The FR10mg/kg, 90min group was given place navigation training at a time at which the induction of LTP in hippocampus was blocked according to the results of Experiment 2. This group was considered to be comparable to the AP5 group in the experiment reported by Morris (1989). It was shown that the acquisition of significant swimming bias as well as induction of hippocampal LTP was inhibited in the AP5 group.

The result was totally different from that of the AP5 experiment. The performance of FR10mg/kg, 90min group in the place navigation training and transfer test was not statistically different from its saline control while AP5 animals were significantly poorer in the respect of escape latency during training as well as the respect of swimming bias during transfer test. Unexpectedly, the performance of the AP5 group was more similar to that of FR10mg/kg, 20min group. The point we should take into account is that the AP5 experiment tested LTP in the same animals that were used in the water maze. Unlike this, Experiment 2 used a naïve and separate group of animals. Experiment 6 was carried out to cope with this point.
In the respect of the timecourse of the drug's effect, the result that the FR10mg/kg, 90min group showed a better performance than the FR10mg/kg, 20min group draws attention. This result suggests the effect of FR115427 on water maze performance is not relevant to its effect on LTP in which the 90 min effect was significantly stronger than the 30 min effect. The timecourse of the effect on learning performance agrees with the timecourse of stereotyped behaviour in the Experiment 1. In addition, the FR10mg/kg, 90min group showed significantly fewer incidents of refused trial than the FR10mg/kg, 20min group. These results suggests that the effect of FR on the spatial learning and on the expressoin of stressed behaviour is more relevant to the effect on spontaneous behaviour than the effect on the hippocampal plasticity.

(c) The performance of MK-801 groups.

It is confirmed that MK-801 does not block the acquisition of spatial memory, represented by the significant bias of swimming in the transfer test, at a dose at which MK-801 does not have any effect on LTP. This results is consistent with the result reported by Halliwell and Morris (1987) and McLamb et al.(1990) (see Table 1-1).

In the present study, even an increased escape latency caused by MK-801 was not clearly detected. It suggests that the drug's effect on the rate of learning acquisition may be variable according to the experimental condition such as the method of training or water temperature. This point is addressed in the following experiment.

Experiment 4 (20 min)

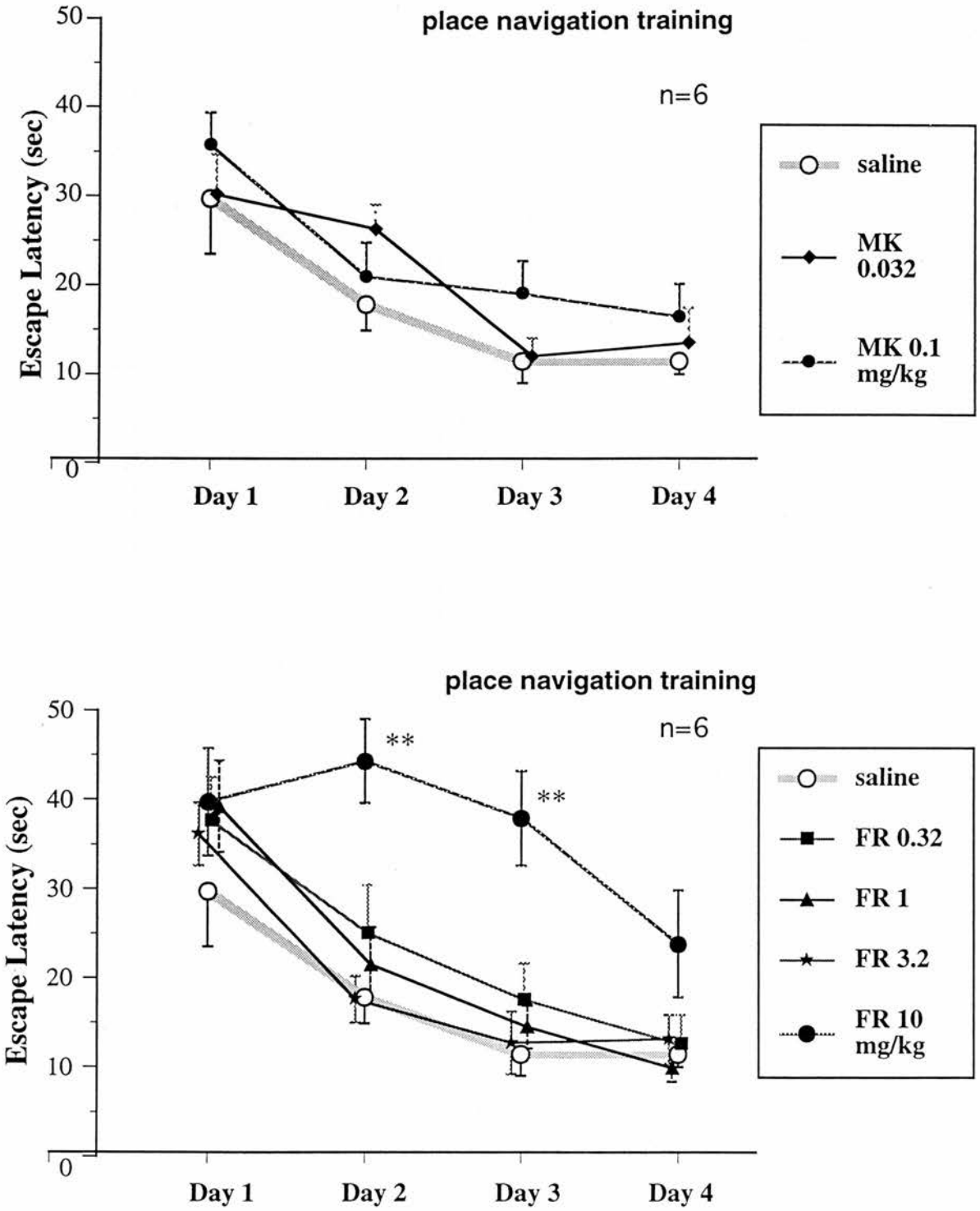
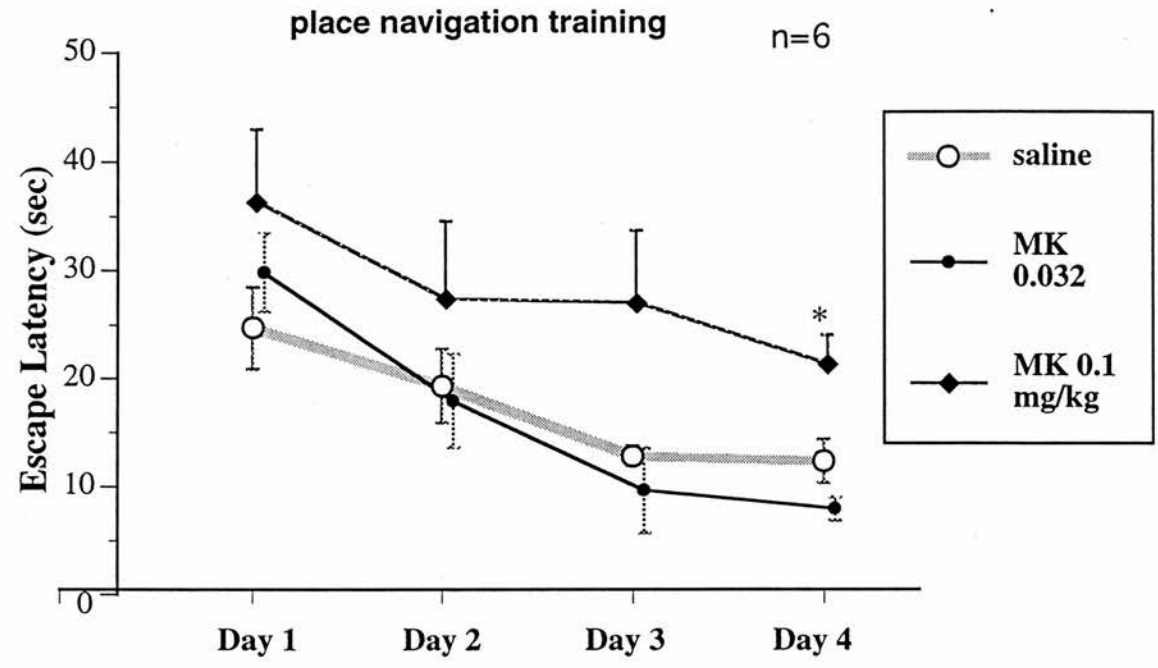


Fig. 6-1 Mean (± s.e.m.) escape latency on each of 4 days training in Experiment 4 (20 min)

** : $p < 0.01$ vs saline group (Dunnett multiple comparison test)

Experiment 4 (90 min)



* : $p < 0.05$ vs saline group (Dunnett multiple comparison test)

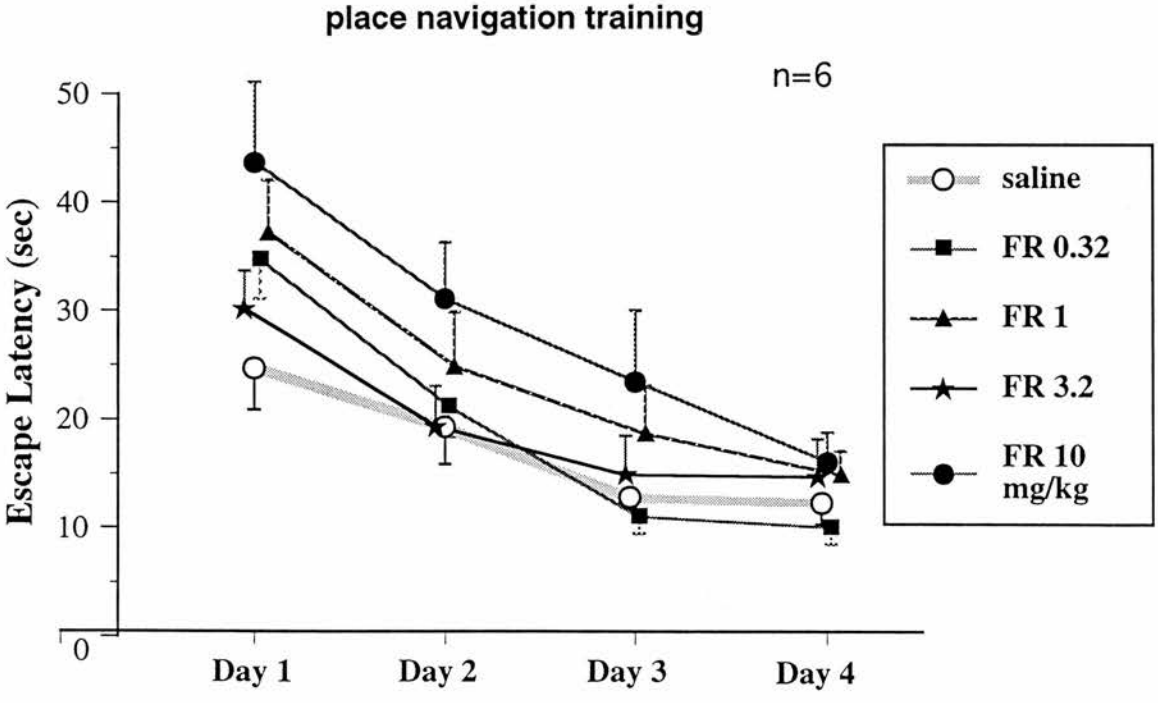
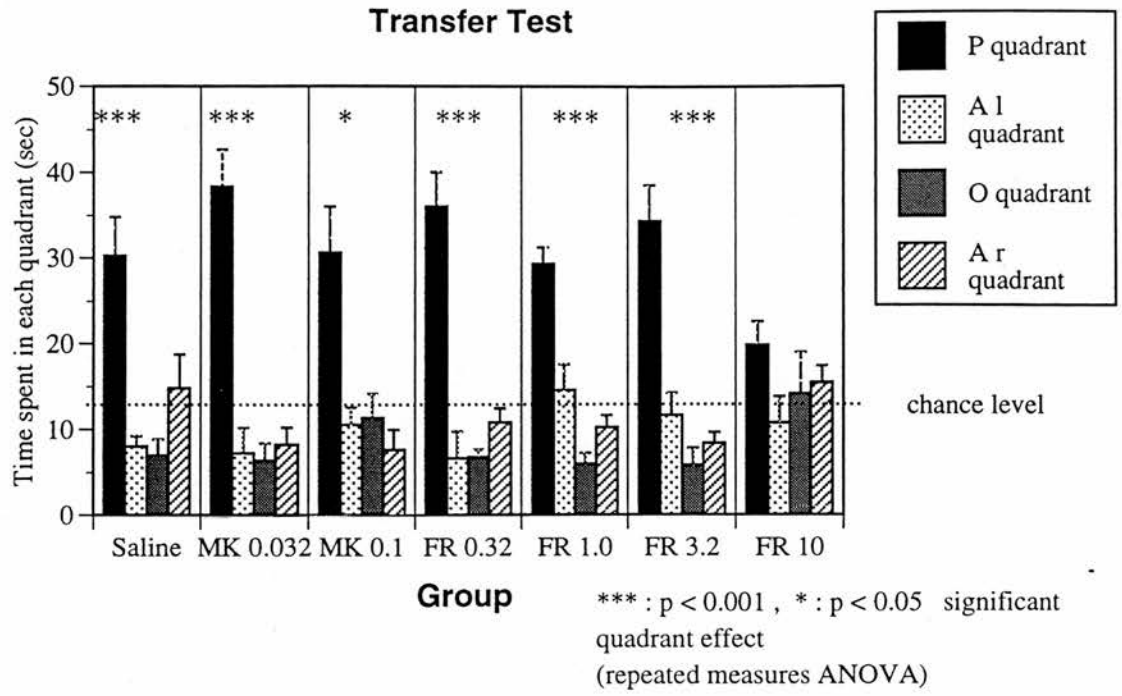


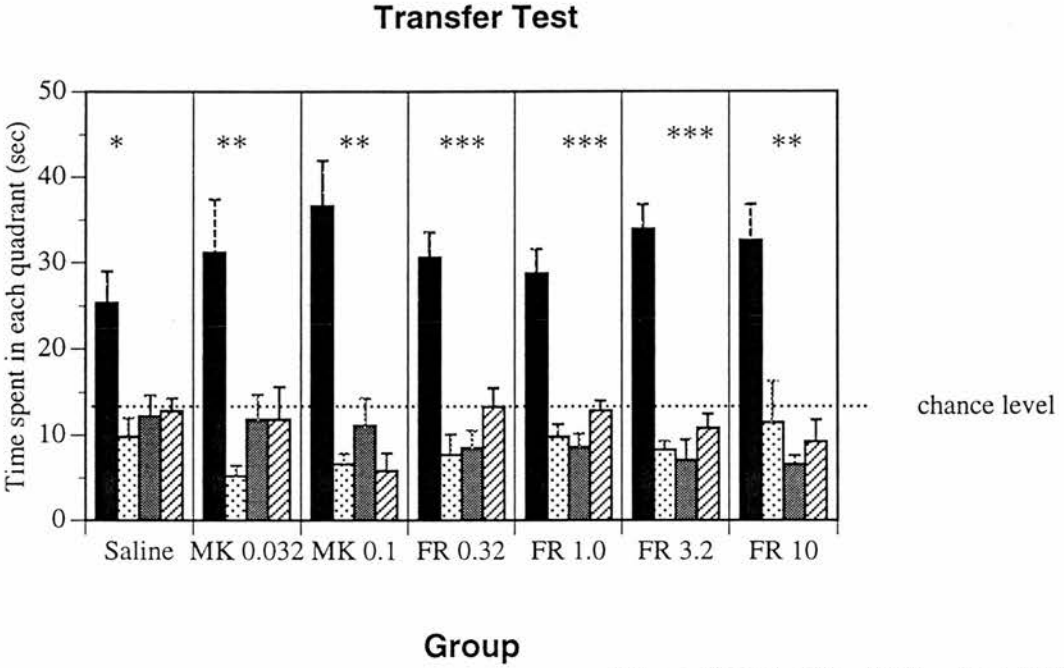
Fig. 6-2 Mean (± s.e.m.) escape latency on each of 4 days training in Experiment 4 (90 min)

(A) Experiment 4 (20 min)



*** : $p < 0.001$, * : $p < 0.05$ significant quadrant effect
(repeated measures ANOVA)

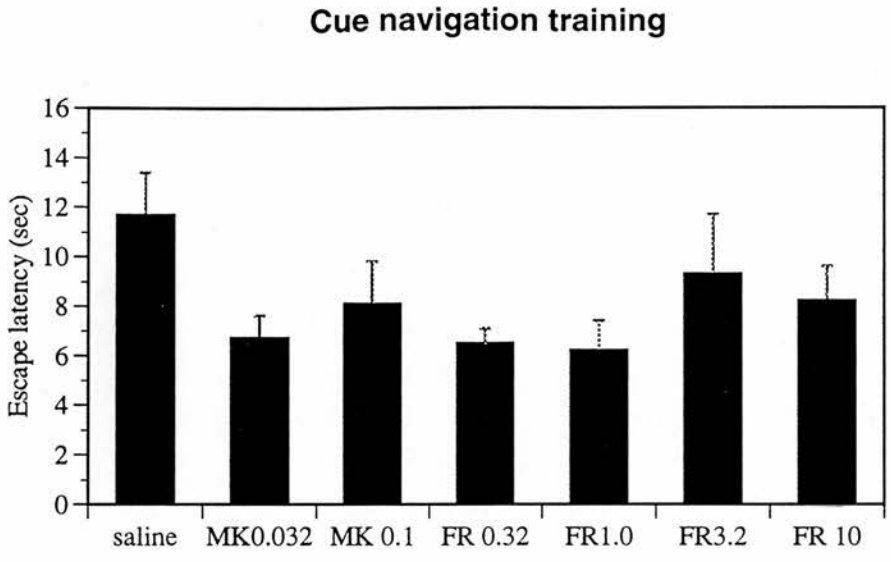
(B) Experiment 4 (90 min)



*** : $p < 0.001$, ** $p < 0.01$, * : $p < 0.05$
significant quadrant effect
(repeated measures ANOVA)

Fig. 6-3 Mean (+s.e.m.) time spent in each quadrant during the 60 sec transfer test on Day 5

(A) Experiment 4 (20 min)



(B) Experiment 4 (90 min)

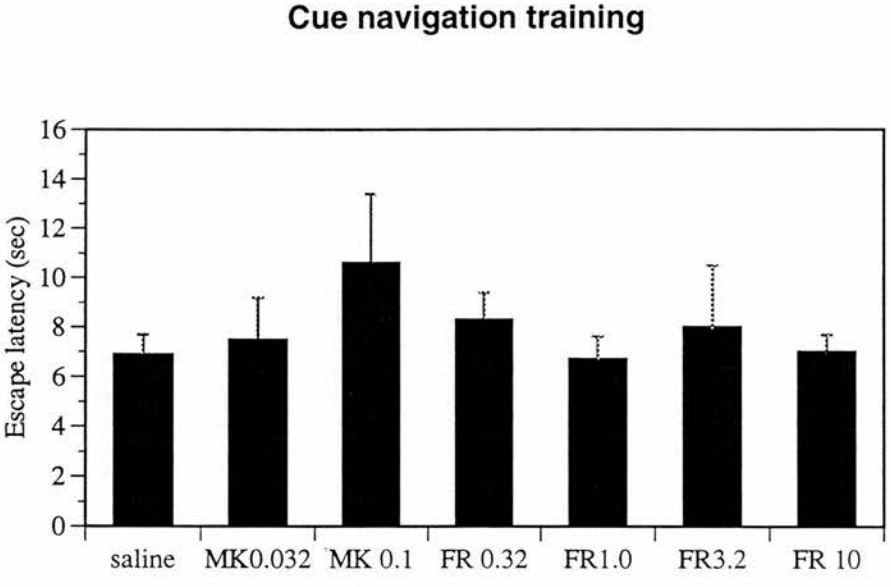
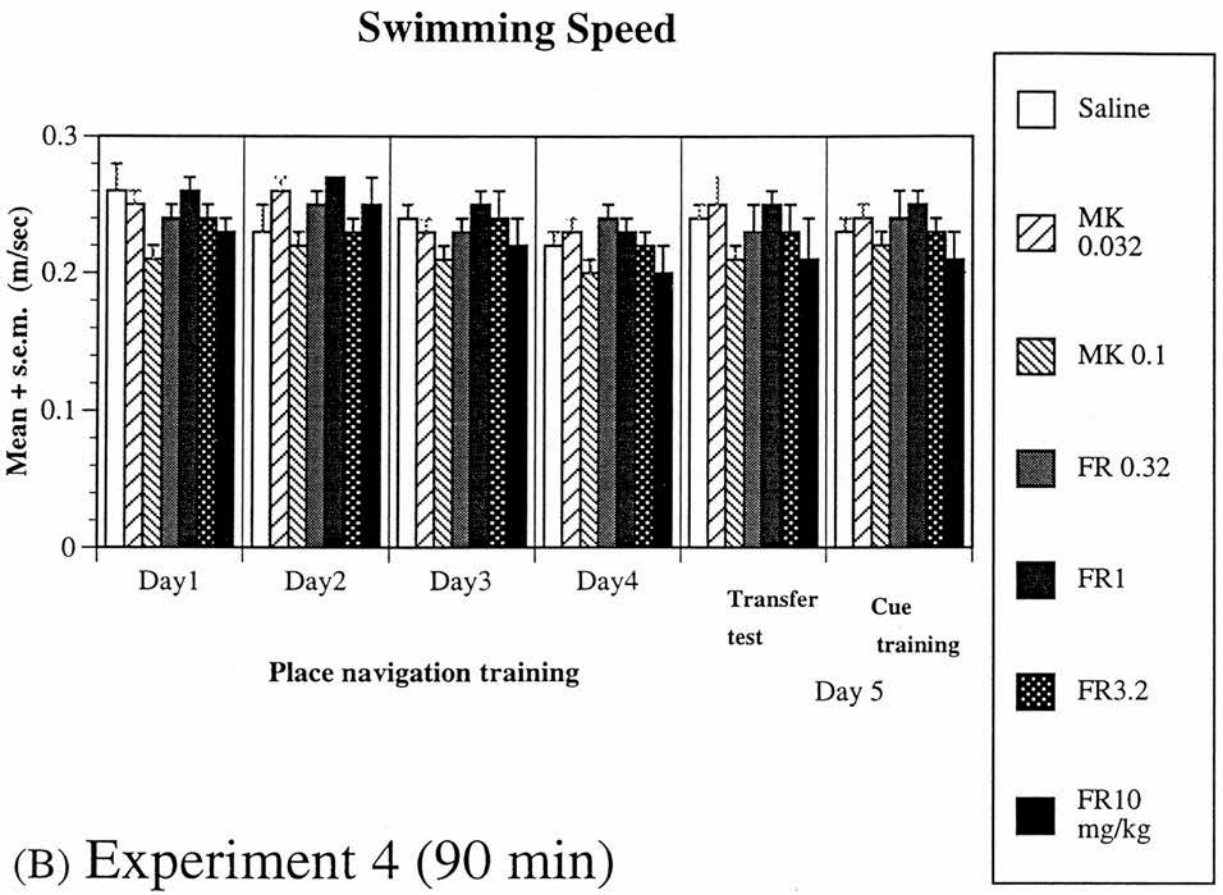


Fig. 6-4 Mean (+s.e.m.) escape latency for 6 trials of cue navigation training on Day 5.

(A) Experiment 4 (20 min)



(B) Experiment 4 (90 min)

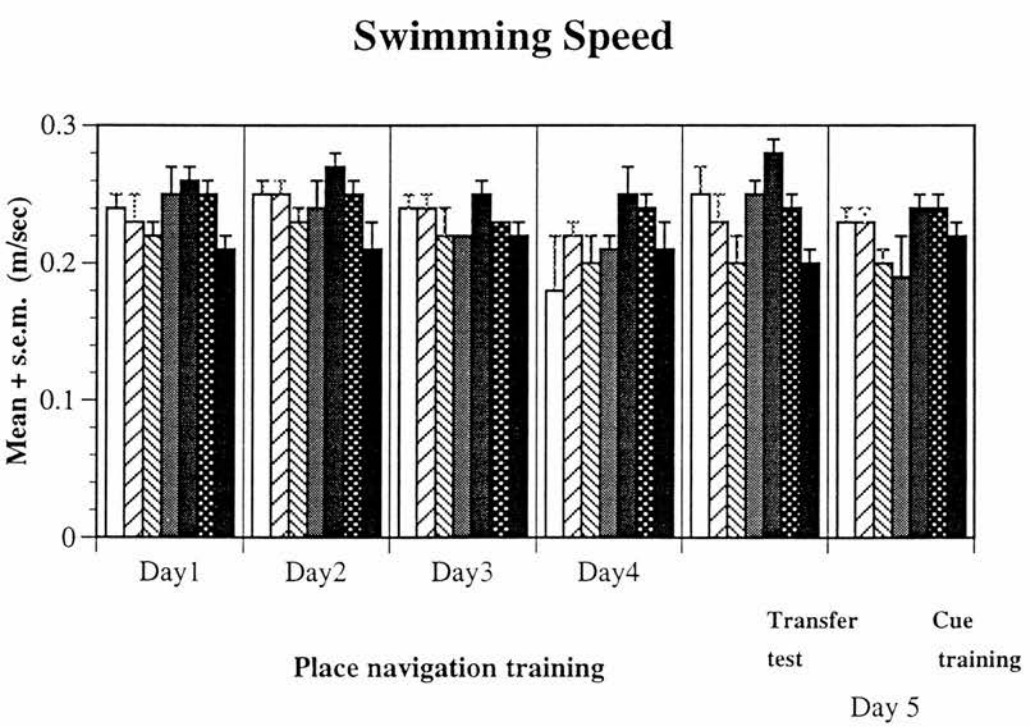
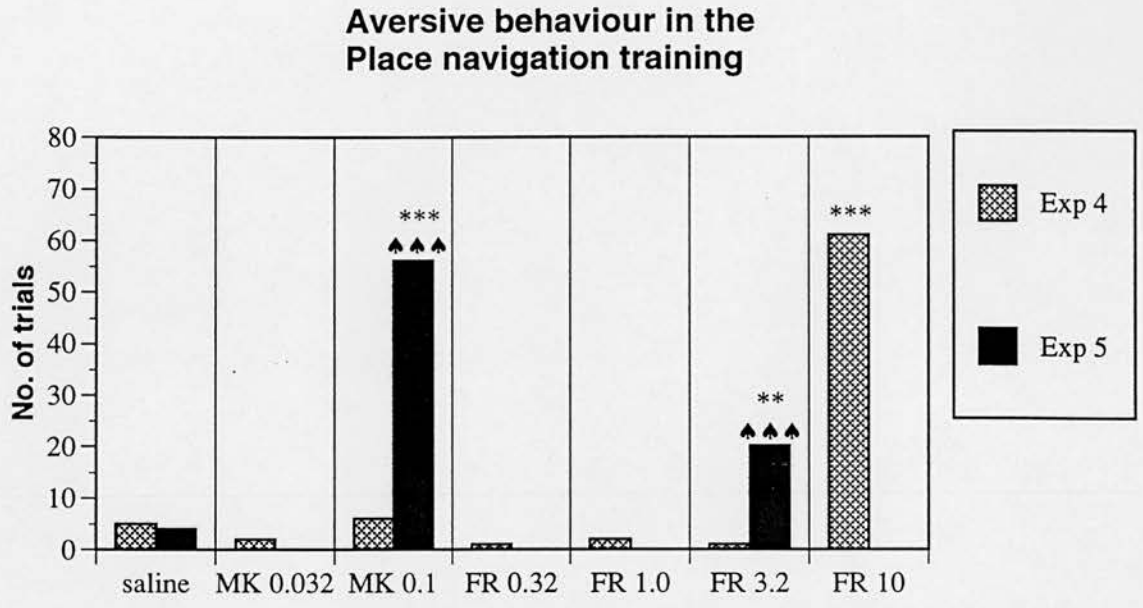
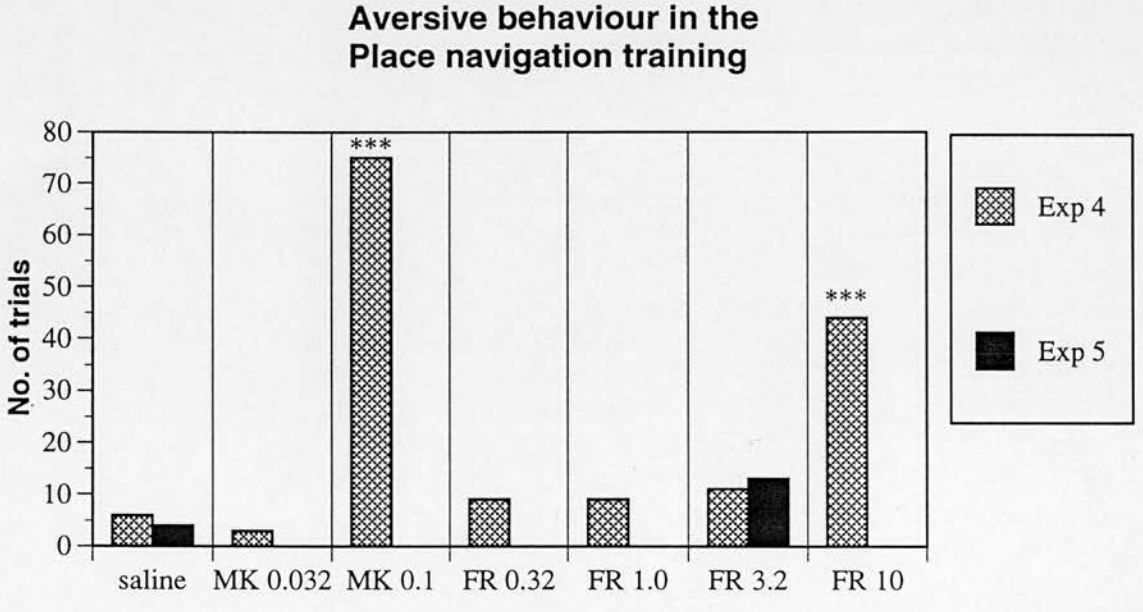


Fig. 6-5 Mean (+s.e.m.) swimming speed during Place navigation training (mean for each day), Transfer test and cue navigation training

(A) Experiment 4, Experiment 5 (20 min)



(B) Experiment 4, Experiment 5 (90 min)



***: p<0.0001, **:p<0.01, *:p<0.05 vs **saline**

♠♠♠: p<0.0001 vs **Exp1** (Fisher's exact test)

Fig. 6-6 Total number of 'refused' trials during place navigation training (Each group had 144 trials in 4 days trainng). The used' trial is a trial on which animals refused to escape onto the submerged platform

6.2 Experiment 5

6.2.1 Introduction

Experiment 5 is a smaller version of Experiment 4 with modification in the experimental procedure.

The purpose of Experiment 5 is to confirm that the impairment of performance induced by FR115427 and MK-801 is dependent on the experimental conditions especially those considered to affect the stress level of animals.

As the FR10mg/kg, 20min animals in Experiment 4 seemed to be too stressed to perform the learning task normally according to their behaviour at the 'refused' trial, it is expected that the reduction of the stress levels in the training (e.g. raising the water temperature, modulation of the procedure of habituation trials) would ameliorate the performance of those groups. However, the relatively poor performance of the control group in Experiment 3 suggest that further reduction of stress level in the present training schedule possibly inhibits the normal learning acquisition. Therefore, an alternative strategy was taken. If the effect of FR115427 on learning is related to stress, a further increase of stress in the experimental condition should deteriorate the performance of drug group.

In order to increase the stress level, the habituation trials were not run, and modulation of the water temperature on Day 1 was removed. As an animal under the influence of drug suddenly experiences cold water, the shock on the first trial is supposed to be much more emphasized than that on the first trial in Experiment 4.

The drug dose used for this experiment was 3.2 mg/kg of FR115427 which had no significant effect on LTP or on learning performance in Experiment 4. The effect at this dose was tested in both 20 min and 90 min interval experiment to test if 20 min effect is stronger than 90 min effect. The effect of MK-801 0.1 mg/kg was also tested

to check whether the effect of MK-801 is also enhanced under these conditions. The interval between injection of MK 0.1 mg/kg and training was fixed to 20 min to compare the result of this experiment with the results of the other reported experiments.

6.2.2 Brief procedure of Experiment 5

The animals received handling 3 times but had no pre-training trials in the first week. The following week, the same training trials and test were carried out as in Experiment 4. The water temperature was fixed at 25.0 ± 0.5 °C throughout the experiment.

6.2.3 Results of Experiment 5

(a) Observation of animal behaviour

As was expected, the number of the refused trial in the place navigation training was increased as compared with Experiment 4 (Fig. 6-6) (page 155). While FR 3.2 mg/kg 20 min interval group showed only 1 refused trial out of 144 trials in Experiment 4, the corresponding group (FR 3.2-20 min) showed 20 refused trials out of 144 in Experiment 5. The Fisher's exact test revealed this increase in Experiment 5 is highly significant ($p < 0.0001$). The number of refused trial of FR 3.2 mg/kg 20 min interval group is also significantly larger than the incidents of control group (4 trials out of 144) in Experiment 5 ($p < 0.01$). The number of incidents in the FR 3.2 mg/kg 90 min interval group in Experiment 5 was not statistically different from that of Experiment 4 (11 trials in Experiment 4 and 13 trials in Experiment 5). A

remarkable increase of refused trial (56 trials) was observed in the MK 0.1 mg/kg group in Experiment 5. The difference between the MK 0.1 mg/kg group in Experiment 4 and the corresponding group in Experiment 5 was highly significant ($p < 0.0001$) and difference between MK 0.1 mg/kg group and control group in the Experiment 5 was also significant ($p < 0.0001$)

No refused trial was observed in the cue navigation training.

20 min interval experiments

(b) Place navigation training

Fig 6-7 (A) (page 162) shows the mean escape latency (± s.e.m.) over 4 days training. Poor performance of FR 3.2 mg/kg and MK 0.1 mg/kg group on Day 2 and Day 3 is noticeable. An over all one-way between and one-way within-subjects ANOVA shows no significant group effect [$F(2,15)=3.217$ $p=0.0688$] and a significant day effect [$F(3, 45) = 33.28$ $p < 0.0001$]. A significant interaction term (group x day) [$F(6, 45) = 2.415$ $p < 0.05$] allows the analysis on each day. ANOVA of escape latency on Day 2 [$F(2, 15) = 4.90$ $p < 0.05$] and on Day 3 [$F(2, 15) = 4.17$ $p < 0.05$] revealed a significant group effect. Post hoc Dunnett multiple comparison test showed that the escape latency of MK 0.1 mg/kg group on Day 2 and escape latency of FR 3.2 mg/kg group on Day 3 is significantly longer than that of saline group ($p < 0.05$ each).

(c) Transfer test

Fig. 6-8 (A) (page 163) shows mean (± s.e.m) time spent in each quadrant during 60 sec transfer test. The saline group failed to show a significant swimming bias to the P quadrant (ANOVA of time spent in 3 quadrants: [$F(7, 10)=2.1904$ $p=0.1626$]). This is caused by a very strange behaviour of one of the saline animals

(record No. 3316). Although it was trained with platform in SW, it showed strong swimming bias to NE (Opposite) quadrant (i.e. 0.1 sec in P, 4.5 sec in Ar, 33.8 sec in O and 21.7 sec in Al quadrant). As it showed normal escape behaviour during training trials, this performance was accidental and mysterious. If the data of this animal is excluded from the analysis, mean swimming time is 31.8 sec in P., 9.5 sec in Ar., 10.2 sec in O. and 8.5 sec in Al. quadrant respectively. An ANOVA of time spent in 3 quadrants shows significant swimming bias [$F(6,8) = 15.71$ $p < 0.01$]. This result is very similar to that of 90 min saline group.

The 3 quadrants ANOVA of the FR 3.2 mg/kg group [$F(7, 10) = 2.14$ $p = 0.1678$] and of the MK 0.1 mg/kg group [$F(7,10) = 3.46$ $p=0.072$] showed no significant swimming bias. Therefore both drug groups failed to acquire the spatial memory.

(d) Cue navigation training

Fig. 6-9 (A) (page 164) shows the performance of each group in the cue navigation training. An ANOVA of mean escape latencies did not show any significant group effect [$F(2, 15) = 0.104$ $p = 0.902$]

90 min interval experiments

(e) Place navigation training.

Fig 6-7 (B) (page 162) shows the mean escape latency (± s.e.m.) over 4 days training. Both groups showed progressive improvement of performance across day. The performance of the FR 3.2 mg/kg group was not statistically different from that of the saline group. A one-way between and one-way within-subjects ANOVA showed that the group effect was not significant [$F(1, 10) = 0.78$ $p = 0.399$]. The main effect

of day was significant [$F(3, 30) = 39.45$ $p < 0.0001$]. The interaction term (group x day) was not significant [$F(3, 30) = 0.90$ $p = 0.454$].

(f) Transfer test

Fig. 6-8 (B) (page 163) shows mean (± s.e.m) time spent in each quadrant during 60 sec transfer test. Both group acquired significant swimming bias to the P quadrant as follow: The 3 quadrant ANOVA in saline group, [$F(7, 10) = 19.71$ $p < 0.001$]; The 3 quadrant ANOVA in FR 3.2 mg/kg group, [$F(7, 10) = 52.95$ $p < 0.0001$].

(g) Cue navigation training

Fig. 6-9 (B) (page 164) shows the performance of each group in the cue navigation training. A t-test shows that the difference between groups is not significant ($t = 0.356$ degree of freedom is 10 $p = 0.7294$)

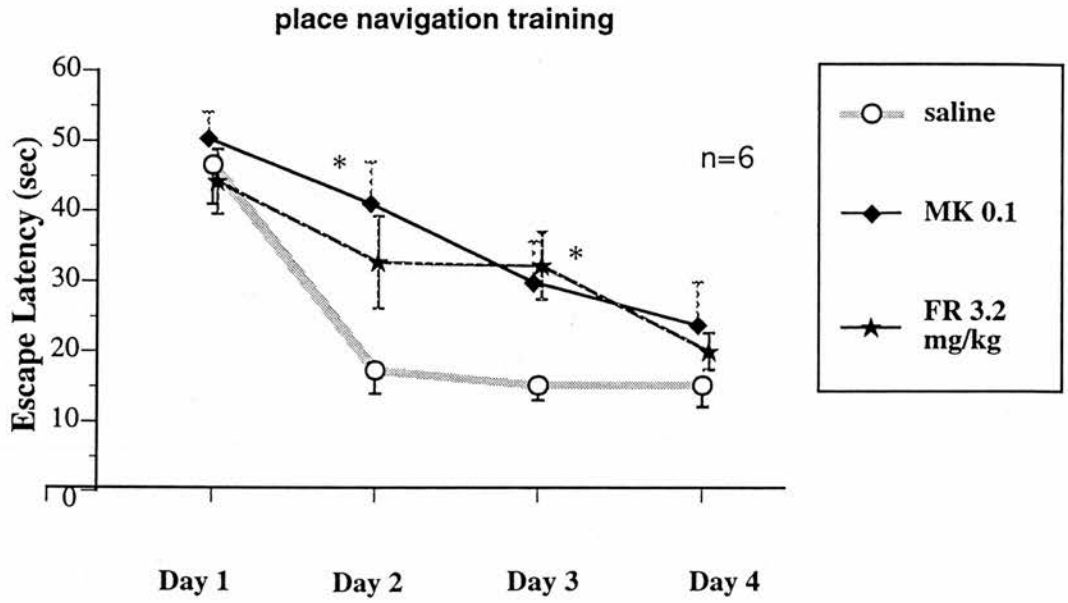
(h) Analysis of swimming speed

The swimming speed of each group during place navigation training, transfer test and cue navigation training is summarised in Fig. 6-10 (page 165). Analysis was carried out on all 5 groups. ANOVA of swimming speed in place navigation training showed no significant group effect [$F(4, 25) = 2.33$ $p = 0.836$], a significant day effect [$F(3, 75) = 5.58$ $p < 0.01$] and no significant interaction between group and day [$F(12, 75) = 0.76$ $p = 0.686$]. An ANOVA of swimming speed in transfer test showed no significant difference among groups [$F(4, 24) = 1.03$ $p = 0.411$] (one swimming speed data of an animal in FR 3.2 mg/kg 90 min group was excluded in this analysis because of a computer problem during the test). An ANOVA of swimming speed in cue training showed no significant group effect either [$F(4, 25) = 0.599$ $p = 0.667$].

6.2.3 Discussion

The performance of FR 3.2 mg/kg 20 min group and MK 0.1 mg/kg 20 min (MK0.1Ex5-I20) group in the place navigation training was significantly deteriorated by reducing water temperature on Day 1 and abolishing the habituation pre-training. However, these modifications did not have a significant effect on the saline or the FR 3.2 mg/kg 90 min group. The enhancement of drug's effect on the learning by the procedural modification seemed to be parallel to the induction of stressed behaviour. The procedural modification increased the number of refused trials in the FR 3.2 mg/kg, 20 min and in the MK0.1mg/kg, 20 min groups but not in the saline or the FR 3.2 mg/kg, 90 min groups. It is suggested that the effect of FR115427 on performance in the water maze is an early onset effect which may be related to the stress in the animals, and unrelated to its late onset effect on LTP. It is not clear whether the effect of MK-801 is early or late onset. However, the nature of its effect on learning was similar to that of FR115427. The performance of the MK0.1mg/kg, 20 min group is comparable to the performance of the MK-801 0.05 mg/kg group reported by Robinson et al. (1989) because significant increase of escape latency (escape path length) and poor biased swimming in the transfer test was observed. The learning effect of FR115427 and MK-801 was not explained by sensory motor impairment because no significant effect was detected in the performance in cue navigation training or swimming speed. To sum up, the effect of these non-competitive NMDA receptor antagonist does not seemed to be closely related to the hippocampal plasticity because those effect are overcome by modulation of stress level in the experimental procedure.

(A) Experiment 5 (20 min)



* : $p < 0.05$ vs saline group (Dunnett multiple comparison test)

(B) Experiment 5 (90 min)

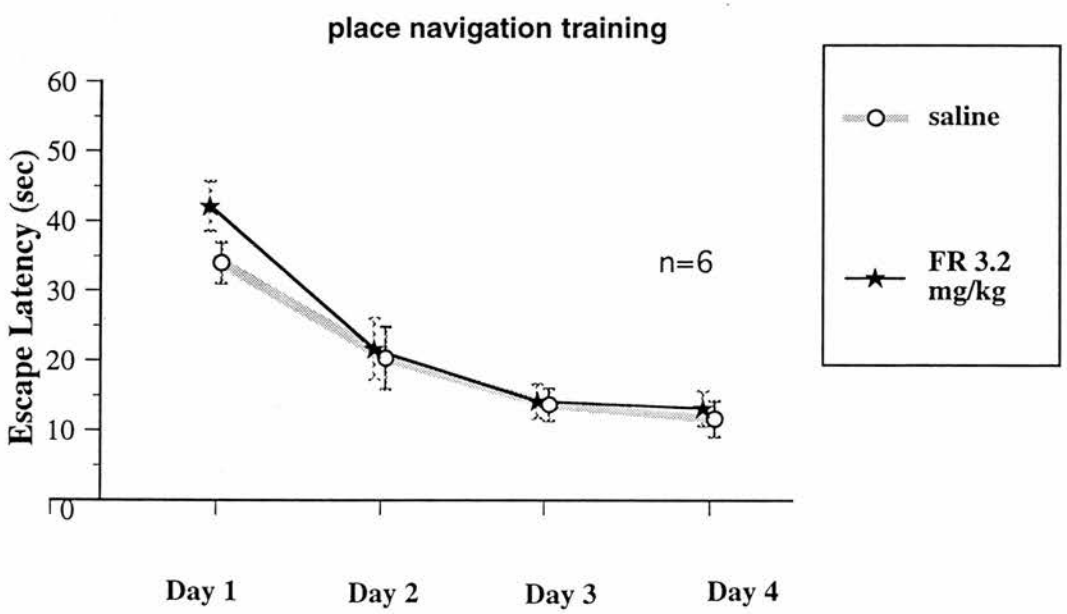


Fig. 6-7 Mean(±s.e.m.w) escape latency on each of 4 days training in Experiment 2 (A) with 20 min interval and (B) with 90 min interval

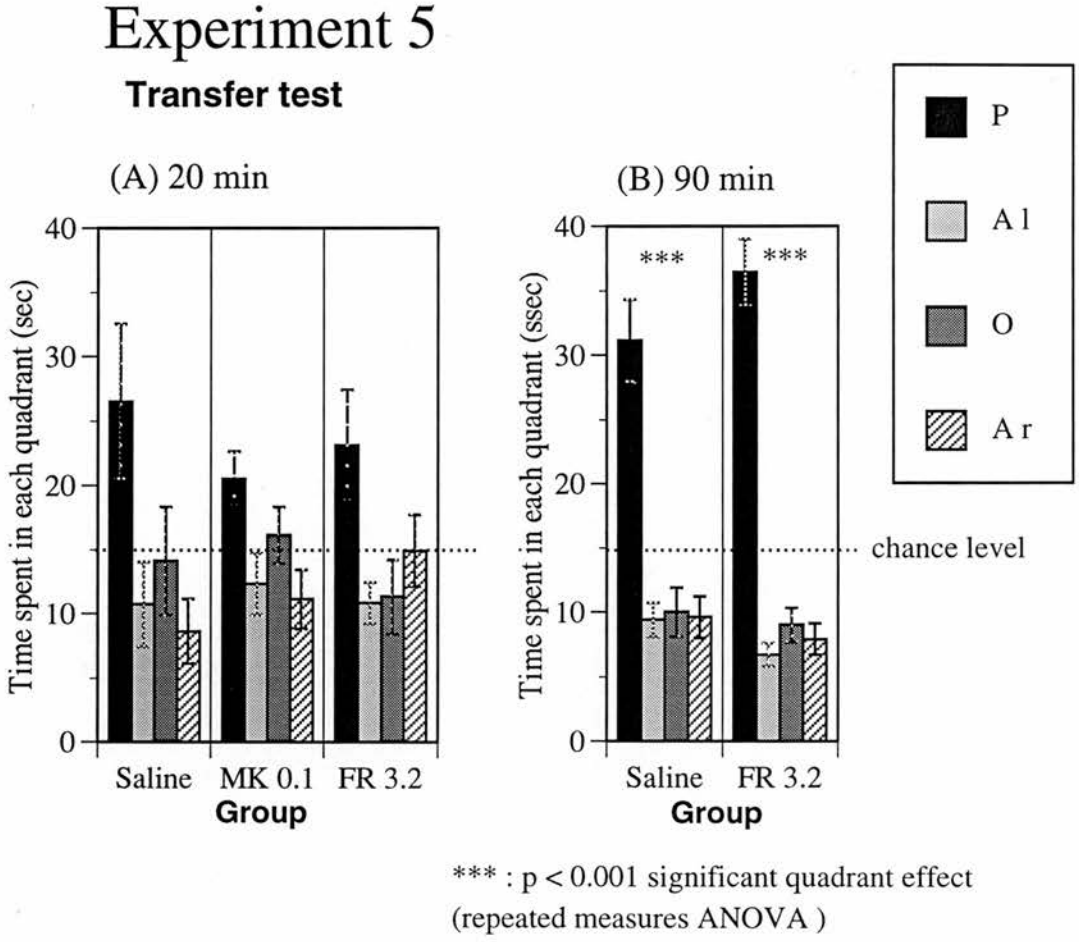


Fig.6-8 Mean (+s.e.m.) time spent in each quadrant during the 60 sec transfer test on Day 5

Experiment 5

cue navigation training

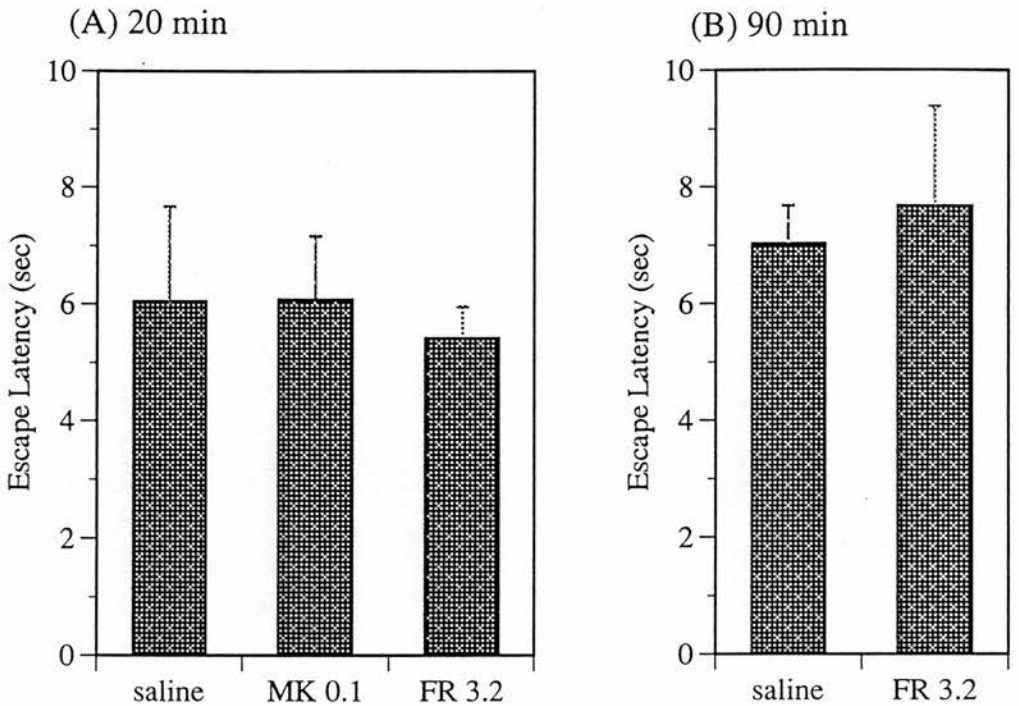
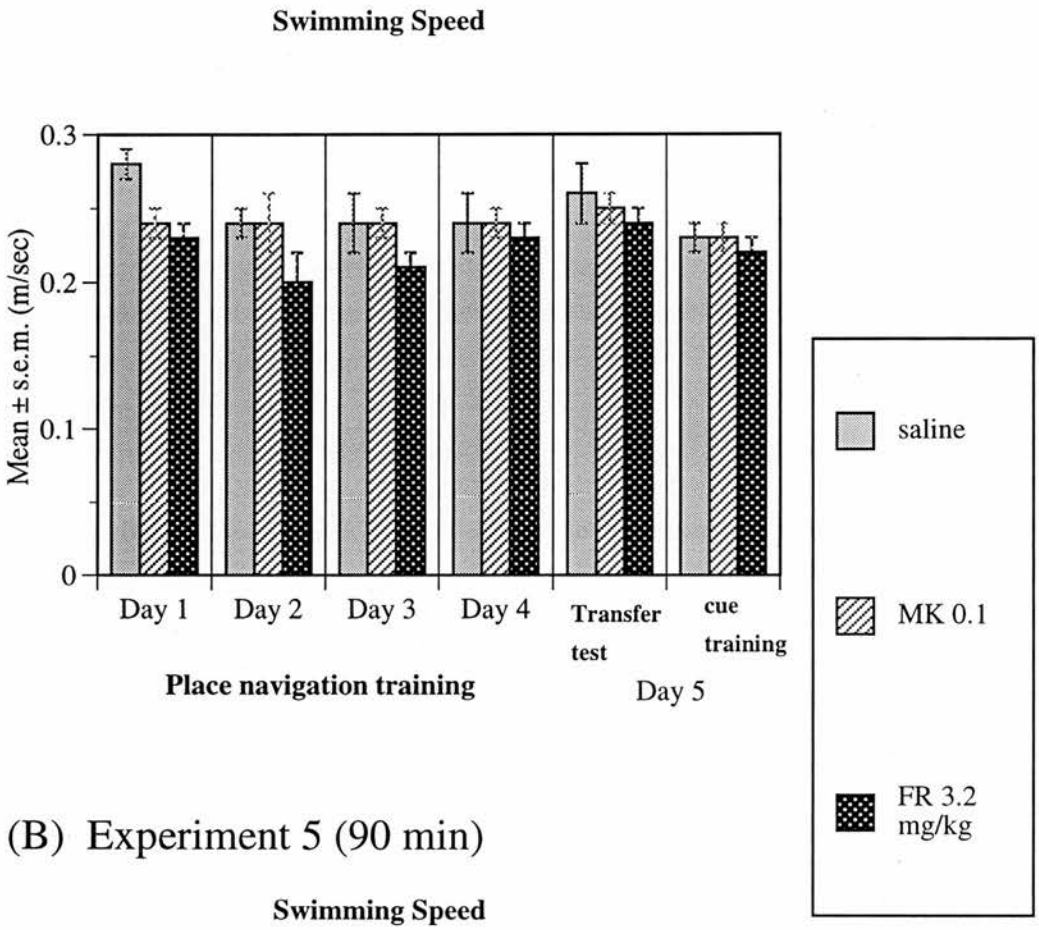


Fig. 6-9

Mean (+s.e.m.) escape latency for 6 trials of cue navigation training on Day 5

(A) Experiment 5 (20 min)



(B) Experiment 5 (90 min)

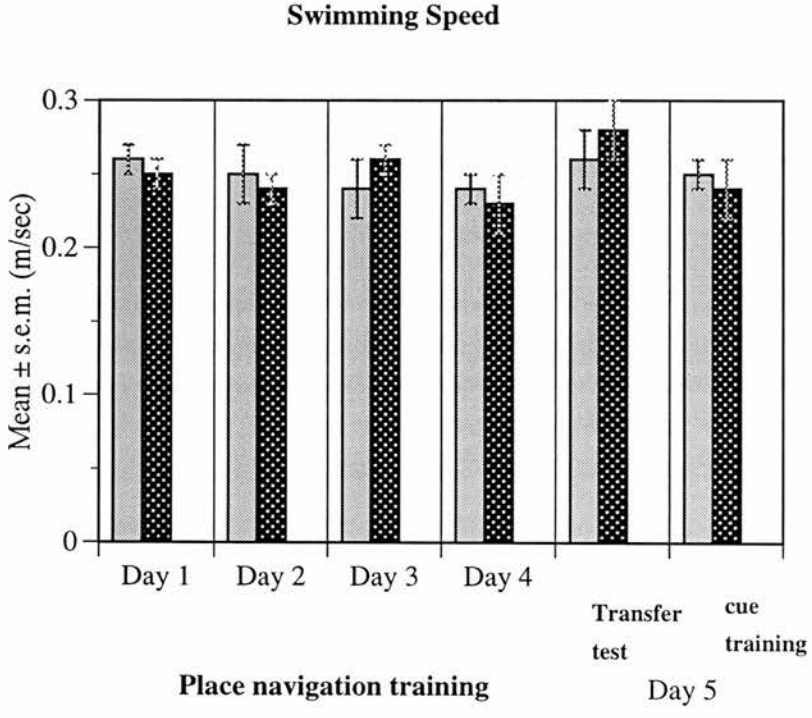


Fig. 6-10 Mean (±s.e.m.) swimming speed during Place navigation training(mean for each day), Transfer test and cue navigation training

6.3 Experiment 6 (LTP experiment)

6.3.1 Methods

On Day 6 of the Experiment 4 or 5, some of the rats which received 3.2 mg/kg or 10 mg/kg of FR115427 during 5 day's training and testing were submitted to Experiment 6. The surgical procedure and electrophysiological method of stimulation and recording are the same as in Experiment 2 (Chapter 3). The animals had received 5 days repetitive treatments of 3.2 mg/kg during the water maze training and received the same 3.2 mg/kg dose of FR in this experiment. The rats that had received 10 mg/kg per day during the training also had the same 10 mg/kg injection in this experiment. The 6th i.p. injection of 3.2 or 10 mg/kg FR115427 was given 30 or 90 min before tetanus stimulation of perforant path.

6.3.2 Results of Experiment 6

(a) Effect of FR on baseline after repetitive injection

As described in Chapter 3, initial EPSP slope of 15 wave forms at the period just before drug injection and the period 25 ~ 30 min after drug injection were averaged and analysed to assess the 6th injection of 3.2 or 10 mg/kg FR115427 on baseline. An one-way within ANOVA revealed no significant change in baseline after 6th injection of 3.2 [$F(1,4) = 6.27$ $p = 0.067$] or 10 mg/kg FR [$F(1,4) = 0.95$ $p = 0.384$].

(b) Effect of FR on LTP after repetitive injection

Tetanus stimulation was administered 30 min or 90 min following 6th injection of FR 3.2 or 10 mg/kg (i.p.). Averages of the initial EPSP slope from 15 wave forms just before and 30 min after tetanus stimulation were made.

Tetanus stimulation 30 min and 90 min after 6th injection of FR 3.2 mg/kg induced a 34.4 ± 5.5 % (n=5) and 25.9 ± 3.2 % (n=5) increase in EPSP slope respectively. Fig. 6-11 (page 169) compares the effect of single injection and 6th injection. These results were not statistically different from the results after a single injection of 3.2 mg/kg FR in Experiment 2.

Tetanus stimulation 30 min after 6th injection of 10 mg/kg FR induced 15.9 ± 1.5 % (n=5) increase in EPSP slope (Fig. 6-11, Fig.6-12(A)).This change was also not statistically different from the result of the single injection in the Experiment 2.

However, tetanus stimulation 90 min after 6th injection of 10 mg/kg FR induced 15.0 ± 5.6 % (n=5) increase in EPSP slope (Fig. 6-11,6-12(B)) while tetanus 90 min after a single injection of 10 mg/kg induced only 5.4 ± 3.1% increase in the Experiment 2. Although the difference between the % change after 6 th injection and after single injection was not significant (t-test , $p = 0.1210$), the tetanus 90 min after a single injection did not induce a significant slope increase in comparison with the pre-tetanus base line ($p < 0.05$) while the tetanus 90 min after a 6 th injection induced a significant potentiation of EPSP slope . In short, in the 90 min interval test, single injection of 10 mg/kg FR blocked induction of LTP but a 6 th injection of 10 mg/kg FR did not block the induction of significant slope increase.

6.3.3 Discussion

At the dose of 3.2 mg/kg FR115427, no difference was observed between the effect of a single injection and a 6 th injection of the drug on LTP.

At the dose of 10 mg/kg, a remarkable difference was observed between the effect of single injection and injection after 5 consecutive treatment. The enhancement of inhibitory effect of the drug on LTP in the 90 min interval test disappeared (see Fig. 6-13 "90 min interval specific tolerance" page 171). This reduction of the drug's effect after repetitive treatment seems to be different from the general desensitisation because the desensitisation was only observed in the 90 min interval effect (if the general desensitisation was induced, the result should be as shown in Fig. 6-13 -"imaginary results"). The mechanism of this unique tolerance is interesting but is unknown. Ligand binding studies suggest that the binding of FR115427 to the NMDA receptor involves more than a simple binding process (Sherriffs et al., 1993). This may be related to the necessity of a longer interval to exhibit the full inhibitory effect on LTP and induction of unusual tolerance. Probably some special events, such as generation of an effective channel block conformation between receptor and ligand, may be induced during the 90 min interval after the initial injection and such a change is blocked after repetitive injection of the drug.

As 10 mg/kg FR 90 min prior to the tetanus did not block the induction of LTP after repetitive injection, it is doubtful that the FR 10 mg/kg 90 min interval group in Experiment 4 was carried out in the condition in which LTP was completely blocked.

As the efficacy of FR115427 in suppressing LTP was not potentiated after consecutive injections, the drug may not be accumulated by daily treatment.

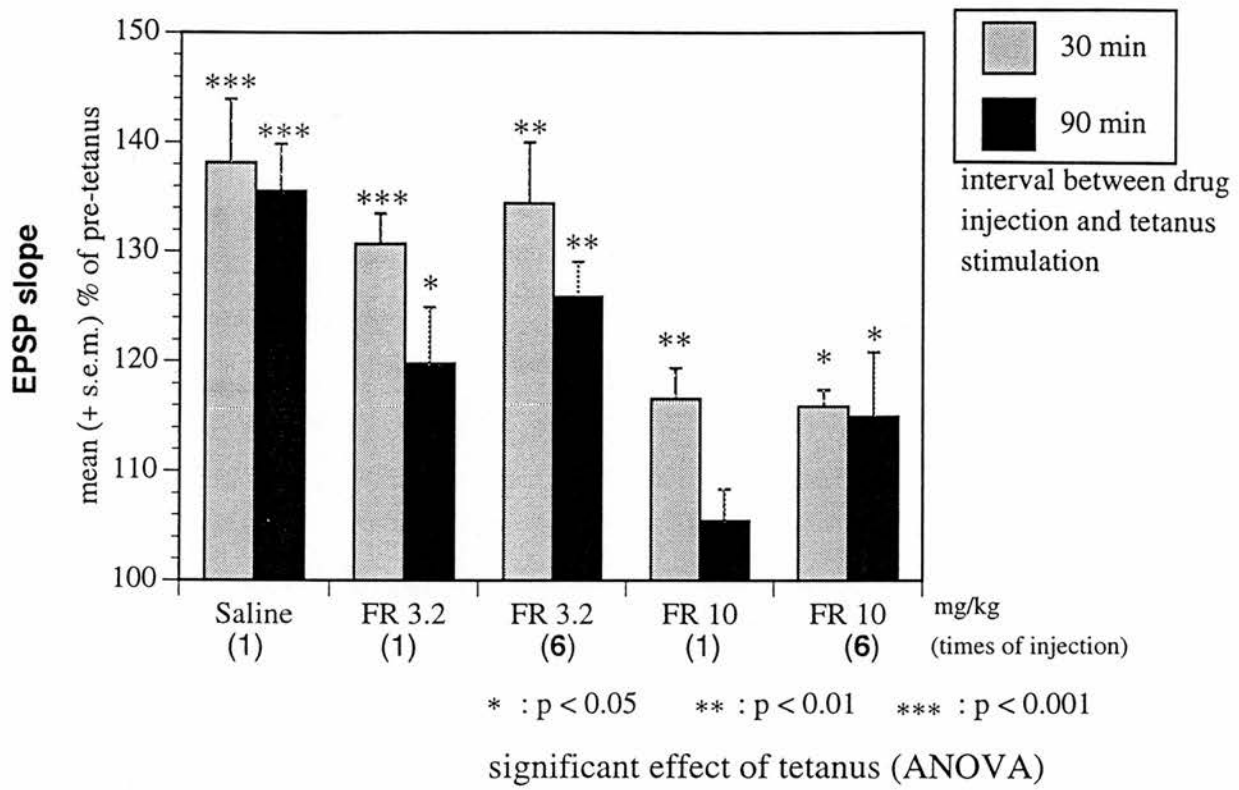


Fig.6-11

The effect of repetitive injection of FR115427 on LTP induction
values are means for 5 min between 30 min and 35 min after tetanus.

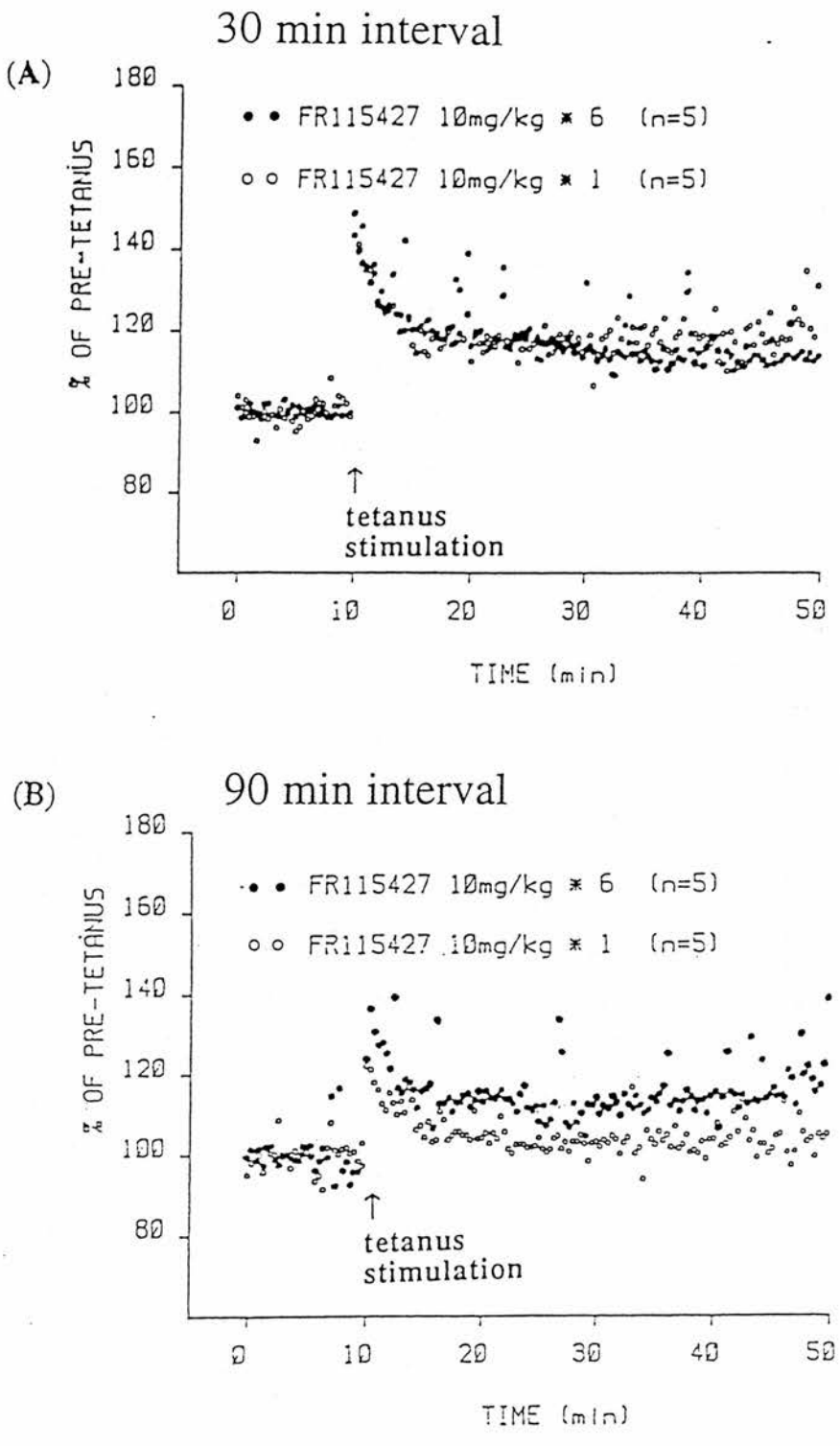
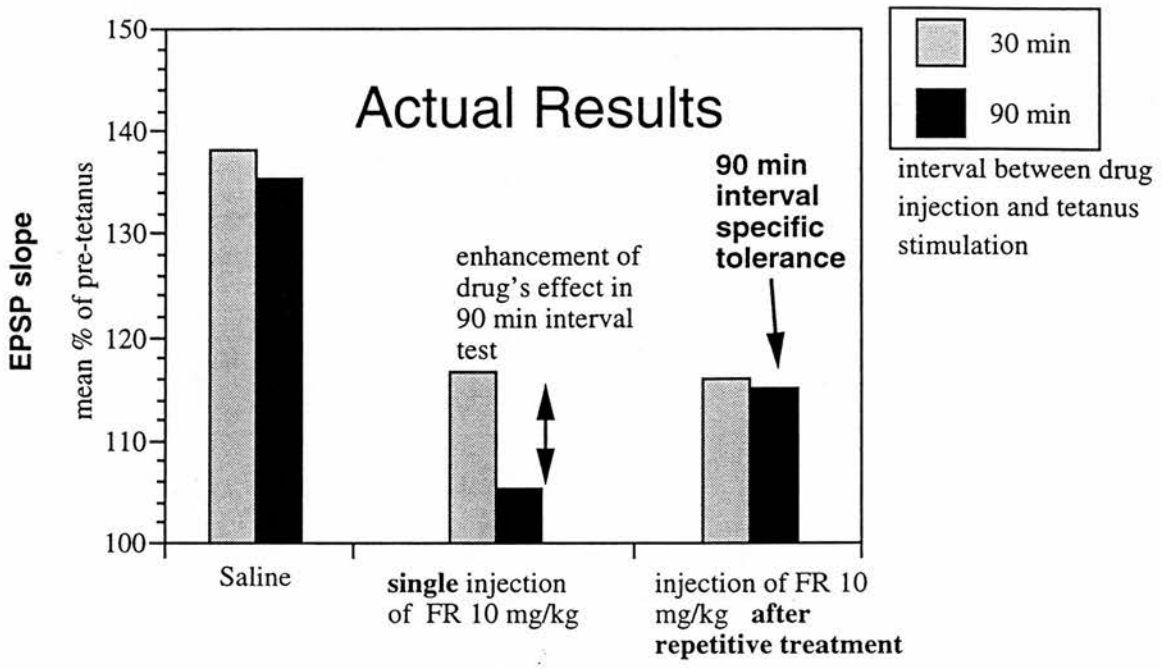


Fig. 6-12

Plots of averaged and normalized EPSP slope value
Plots were made between 10 min before and 40 min after tetanus stimulation
In(A), tetanus stimulation was applied 30 min after drug injection (i.p.).
In(B), tetanus stimulation was applied 90 min after drug injection (i.p.).

Close dots show the result after single injection of drug.
Open dot show the result after 6 th injection of drug.



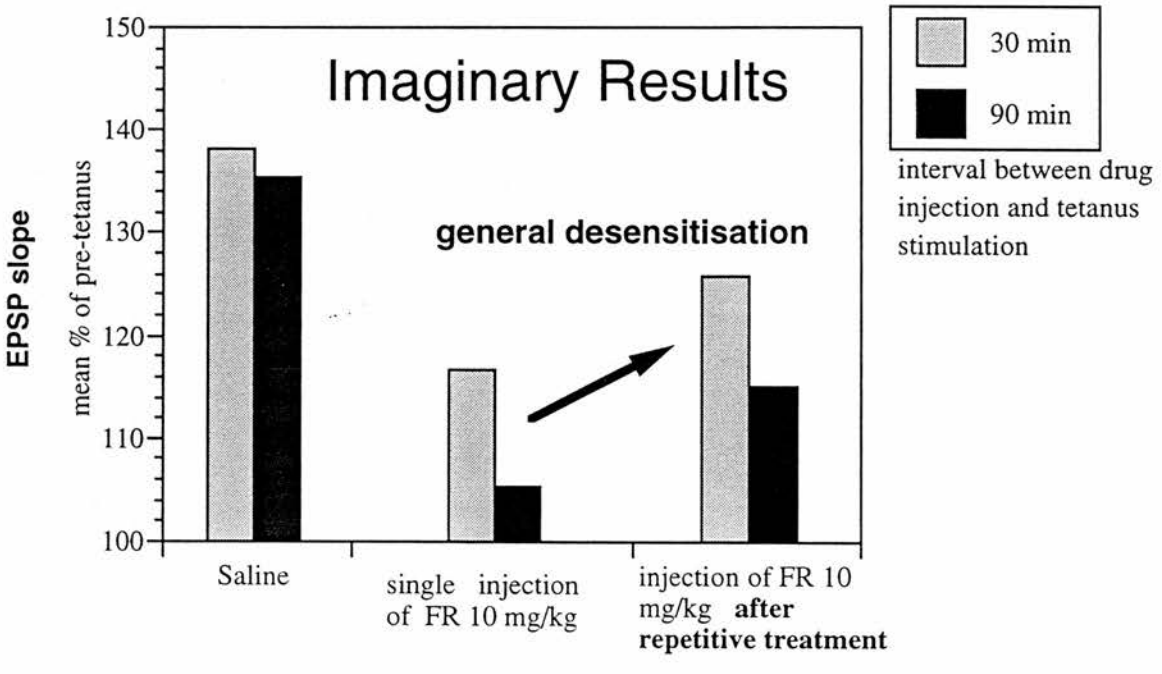


Fig. 6-13
The effect of repetitive injection of FR115427 on LTP induction

Chapter 7
General Discussion

The aim of this thesis has been to examine whether the effect of FR115427 on spatial learning and on LTP show similar dose and time dependency. If they are similar, results strongly support the hypothesis that LTP is a form of neural plasticity underlying spatial memory. If they are not similar, results suggest that NMDA receptor mediated process which is different from LTP plays an important role during learning performance, standing alongside the LTP hypothesis. The results which were obtained in the present experiments supported the latter suggestion. These experiments also confirmed that the induction of motor syndrome is another example of behavioural effects of the NMDA receptor antagonists which can be distinguished from the effect on LTP. At first, this general discussion summarises the experimental results show the effect of FR115427 on behaviour and learning to be different from its effect on LTP, then an analysis of the process the NMDA receptors are involved in during learning in the water maze.

7.1 Summary of results

This thesis consists of 6 experiments (Experiment 1 ~ 6). The animals utilised in Experiment 6 were the animals already tested in Experiment 4 and Experiment 5 while Experiments 1 ~ 5 utilised naïve animals. All drug treatments were done by i.p. injection.

7.1.1 Experiment 1: Open field activity study

FR115427 and MK-801 induced a characteristic motor syndrome: ataxia, head weaving, body rolling and hyperlocomotion. The minimum effective doses of FR115427 were 32 mg/kg for induction of ataxia or body rolling and 10 mg/kg for the induction of head weaving or hyperlocomotion. This potency was 30 ~ 100 times weaker than that of MK-801. Scores of Ataxia and head waving reached a peak within 30 min after injection of FR. The time course of hyperlocomotion and body rolling seemed to be exhibiting a similar time course to the dose at which ataxia was not very severe. The behavioural effects of MK-801 appeared slower than that of FR115427.

7.1.2 Experiment 2: *in vivo* LTP experiment

FR115427 suppressed the LTP (sustained increase of slope EPSP induced by tetanus stimulation of perforant path) in the dentate gyrus of anaesthetised rats dose dependently. An analysis of the effect of FR115427 on LTP at different doses and at different post injection periods showed that the drug's effect is significantly more potent after a 90 min than a 30 min interval following injection. The induction of LTP was completely blocked 90 min after injection of 10 mg/kg dose of FR115427 but not after 30 min. MK-801 exhibited no significant effect at the dose of 0.32 mg/kg or 0.1 mg/kg. All anaesthetised animals died after injection of 1 mg/kg dose of MK-801 in the present experiment using Lister hooded rats. It is reported that 1 mg/kg MK-801 completely blocked LTP induced 150 min or 120 min after injection but not 30 min after injection in anaesthetised SD rats (Abraham and Mason, 1989; Morimoto et al., 1991). Both the observed effect of FR115427 and the reported effect of MK-801 on

LTP were enhanced after the longer interval . This time course effect of FR115427 is totally different from the behavioural effect. This potency of FR115427 is about 10 times weaker than the reported potency of MK-801.

7.1.3 Experiment 3: the water maze experiment with hippocampal lesion animals (in mild stress version)

The conventional procedure of the water maze was modified in this experiment using habituation trials before the main training with a hidden platform and warmer water on the first day of the main training. These procedural modifications were conducted to alleviate the stress of Day 1 training. This experiment's aim was to examine whether the spatial learning under conditions of mild stress depends on the same hippocampal functions as learning under the conventional condition.

After 4 days training (1 day training in the warmer water: 27 °C, followed by 3 days training in the normal water: 25 °C), sham animals succeeded but hippocampal lesion animals failed to acquire the spatial memory as exhibited by the bias seen in the swimming pattern in the transfer test. These results showed that spatial learning in the modified procedure was still sensitive to the hippocampal damage. The performance of hippocampal lesion animals in cue navigation training suggested that their learning deficit was not due to side effects of the lesion surgery on their sensory motor system.

7.1.4 Experiment 4: water maze experiment (mild stress)

In Experiment 4, the same procedural modification (habituation trials and modulation of water temperature) were introduced.

The 10 mg/kg dose of FR115427 interfered with the acquisition of spatial memory if a 20 min interval was taken between drug injection and commencement of training, but had no significant effect if a 90 min interval was taken (training took about 10 min each day). At the dose of 3.2 mg/kg or lower doses, FR115427 had no effect, with either the 20 or 90 min interval. MK-801 had no significant effect at 0.1 mg/kg or lower doses. Both drugs did not induce significant effect on performance in the cue navigation training.

While FR more strongly suppressed LTP after longer interval (90 min, 150 min) than after the shorter interval (20 min, 30 min), learning after the short interval (20 ~ 30 min) was severely impaired but no impairment was observed after the longer interval (90 ~ 100 min).

The above results suggested that the time course of the learning effect is different from that of the inhibitory effect on LTP.

7.1.5 Experiment 5: water maze experiment (normal stress)

In the Experiment 5, the habituation trials and modulation of water temperature were removed. The 3.2 mg/kg dose of FR impaired the acquisition of spatial memory in the 20 min interval training, but did not impair memory acquisition in the 90 min interval training. The 0.1 mg/kg dose of MK-801 impaired the acquisition of spatial memory in the 20 min interval training.

This experiment suggested that FR's effect on learning and LTP were different in the dose response as well as in the time course. The 3.2 mg/kg dose of FR did not significantly suppress LTP in Experiment 2 but it did impair learning in this experiment. MK-801 also impaired the spatial learning at a dose (0.1 mg/kg) that did not block LTP.

Another interesting finding was that the potency of those drugs on learning seemed to depend on the level of stress to which the animals were exposed on the first day of training. The deterioration of the learning performance in the FR group and MK group in Experiment 5 seemed to be proportional to the significant increase of 'refused' trials in this experiment . Neither FR nor MK impaired performance in the cue navigation training. FR115427 and MK-801 were found to exhibit similar effects on learning.

7.1.6 Experiment 6: LTP experiment after water maze experiments

Following the water maze experiments, the effect of FR115427 on LTP after the daily repetitive injection was examined. While single injection of 10 mg/kg FR115427 blocked LTP induced 90 min after injection, a 6th injection of 10 mg/kg FR after 5 consecutive injections did not completely block LTP induced 90 min after injection. The drug's efficacy of suppressing LTP induced 30 min after injection was not changed by repetitive injection. An apparent desensitisation effect specific to the 90 min interval (using FR115427) seemed to occur after repeated treatment. No noticeable change was detected in the drug's effect after repetitive injection of the 3.2 mg/kg dose. The possibility that the accumulation of drug potentiated the drug's effect was excluded.

On the other hand, it was found that even the highest drug dose group in Experiment 4 was not always in the equivalent condition in which LTP was completely blocked. As an attempt to examine learning performance of defective animals in hippocampal LTP failed, the above results do not deny the LTP hypothesis.

7.2 The nature of the effect of non-competitive NMDA receptor antagonists on learning

In Experiment 4 and 5, three groups showed a significant deficit in learning performance. They are as follows: the FR 10 mg/kg 20 min interval group in Experiment 4, FR 3.2 mg/kg 20 min interval group in Experiment 5 and MK 0.1 mg/kg 20 min interval group in Experiment 5. For convenience, these groups shall be termed FR10Ex4, FR3.2Ex5 and MK0.1Ex5 respectively. One remarkable point is that equivalent groups in Experiment 4 to groups FR3.2Ex5 and MK0.1Ex5, which are FR3.2Ex4 and MK0.1Ex4 in the 20 min interval experiment, showed no impairment in their learning performance. That is to say, the procedural manipulation in the Experiment 4 canceled the drug's effect on learning observed in the FR3.2Ex5 and MK0.1Ex5 group. This point seems to be the key to understand the nature of the learning effect of FR115427 and MK-801.

7.2.1 Memory consolidation/retention process and memory retrieval process

The procedural modification of the Experiment 4 was introduced in the very early stage of learning (before training and during training on Day 1). On the other hand, the process for consolidation and retention of the acquired memory during training (spatial information of the hidden platform) and the process for the retrieval of that memory may not be active until the end of training on Day 1 or later. Therefore, it is suggested that the learning effect of FR or MK is not caused by the impairment of the memory consolidation, retention or the memory retrieval process. As for MK-801, some groups confirmed this point by modulating the timing of drug injection.

Robinson et al. (1989) showed that administration of MK-801 immediately after place navigation training of each day, did not interfere learning (exhibition of a significant swimming bias in the transfer test) at the dose (0.1 mg/kg). McLamb et al. (1990) obtained the similar results. These provide groundwork for the argument that MK-801 does not interfere the memory retention/consolidation process.

Robinson et al. (1989) and Heale and Harley (1990) revealed that MK-801 does not block the retrieval process of memory along to behavioural expression of memory using the 'place recall' test (see Chapter 1). In that test, animals treated with the vehicle were trained in advance and MK-801 was injected only before the transfer test. Since MK-801 did not disrupt the expression of the acquired memory, it had no effect on memory retrieval.

7.2.2 Learning acquisition process

The only remaining process of learning which can be impaired by FR115427 or MK-801 is the acquisition process of learning. However, the main process of learning acquisition, an acquisition of substantial 'information' about platform location, should take place throughout the training period. If this process is impaired by a drug, it becomes difficult to reverse the impairment, ever using circumstantial modifications at the only beginning of the training. In order to explain the cancellation of the drug's effect by the modification at the initial stage, one has to presume some early stage of the learning acquisition process which is crucial to the following process.

The poor learning groups; FR10Ex4, FR3.2Ex5 and MK0.1Ex5 seemed to have poor motivation to escape to the platform because they showed high incidence of 'refused' trial in the early stage of training. They seemed to have troubles in initiation

of substantial learning. This observation suggests the existence of process which is essential to initiate the learning. Whishaw et al. (1995) called the establishment of learning constitution "finding the solution". Hence, the acquisition of learning processes can be subdivided into 2 stages; (i) 'finding the solution' process and (ii) 'learning the task' process.

For example, this idea of subdivided acquisition process is explained as follows. In order to acquire the normal escape performance in the water maze, (i) a rat has to understand that memorising the position of submerged platform is beneficial to them at first, (ii) then it has to organise and memorise the 'information' which is necessary to specify the platform's location. Only if the submerged platform is satisfactory for a rat to escape, (i) process may be accomplished. However, the submerged platform may not be necessarily a satisfactory refuge for a rat which is strongly aversive to water as it does not enable rats to get out of the water completely. Strong aversion may force a rat to continue to search a better foothold or hidden path leading to a complete escape. Conversely, if swimming in the water is not an aversive event, they may not try to reduce the escape latency by memorising the position of platform. They may prefer to take an easygoing random search strategy. The 'finding the solution' process can be understood as an acquisition process of the proper attitude to the escape platform and it seems to be influenced by psychological states of animals.

Going back to the initial point of argument of this section, if there is an inhibition of learning process which can be abolished by circumstantial modifications in the early stage of learning, the only possible inhibition may be an inhibition of learning process (i) but not (ii). FR115427 and MK-801 seem to prevent animals from learning that the submerged platform is worth to escape or to memorise its location.

7.2.3 Learning effect of nitric oxide synthase inhibitor.

A report by Bannerman et al. (1994a, 1994b) which studied the effect of L-nitro-arginine methyl ester (L-NAME), a nitric oxide synthase inhibitor, on LTP and spatial learning gives a good clue about a role of NMDA receptors in learning process. Inhibitors of nitric oxide synthase are expected to share some of the physiological actions with NMDA receptor antagonists because those inhibitors not only block induction of LTP (Bohme et al., 1991; O'Dell et al., 1991; Schuman and Madison, 1991; Haley et al., 1992) but also attenuate some stimulatory effects mediated by NMDA receptor such as, formation of cyclic GMP in rat cerebellar slices (Bredt and Snyder, 1989; East and Garthwaite, 1990) or in hippocampal slices (East and Garthwaite, 1991) or in cerebellum of free moving rats (Luo et al., 1994), release of noradrenaline and aspartate from rat hippocampal slices (Jones et al., 1995) and excitotoxic damage in rat cerebral cortical culture (Dawson et al., 1991) .

Similar to the results of present experiments, Bannerman et al. showed that L-NAME produced an impairment in spatial learning in the water maze at a relevant dose at which it did not block *in vivo* LTP. More interestingly, they found that once rats have experienced a water maze task in the normal condition, L-NAME did not impair the learning in a second water maze located in a novel spatial environment. In another words, L-NAME did not block spatial learning in rats which were already familiar to aspects of the water maze but had not acquired any of the spatial information about the new maze. In the first water maze, rats seemed to acquired something essential but different from the substantial information to solve the second maze. This acquisition may be the acquisition of process (i). This process may be an acquisition of a proper regard to the platform as a satisfactory refuge for rats or a proper choice of place navigation strategy in solving the maze. L-NAME, FR115427 and MK-801 blocked acquisition of process (i) but could not reverse or cancel the acquired process (ii).

7.3 Anxiety and water maze

In the present Experiments, FR115427 and MK-801 increased the incidents of 'refused' trial which seemed to be an expression of aversion to water. As the reduction of water temperature increased the incidents of 'refused' trials and the increase in the FR3.2Ex5 and MK0.1Ex5 group paralleled their poor performance; too strong stress in the water seemed to prevent rats from regarding the position of platform as a worth place to memorise.

Matching to the above point of view, too weak stress is also considered to prevent rats from regarding the position of platform as a worth place to memorise because rats have weak necessity to reduce the escape latency by memorising the position of platform. Consistent to this view, anxiolytics were reported to impair water maze learning. Chlordiazepoxide (McNaughton and Morris, 1987), morphine (McNamara and Skelton, 1991a), buspirone (McNaughton and Morris, 1992), diazepam (McNamara and Skelton, 1991b; 1992a) and CL218,872 (McNamara and Skelton, 1992b) impair learning in the water maze. Despite the different structure and different side effects, they all have anxiolytic effects in the behavioural tests. Interestingly, McNamara and Skelton (1991a) reported that not only naloxon reverse the acquisition impairment caused by morphine, reducing the water temperature also reversed the effects of morphine. Reducing the water temperature canceled the effects of anxiolytics. This result shows a fine contrast to the results of present experiments, in which increasing water temperature canceled the effect of FR115427 or MK-801 that induced stressed behaviour in the water maze. Therefore the anxiogenic aspect in the effect of FR115427 and MK-801 is suggested to be important in the induction of learning effect in the water maze.

However, anxiolytic-like properties of competitive and noncompetitive NMDA receptor antagonists have been generally demonstrated in rodent models, including

social interaction, elevated plus-maze (Dunn et al., 1989), separation-induced ultrasonic vocalisation (Kehne et al., 1991), and conflict paradigms (Bennett and Amric, 1986; Cineschmidt et al.,1982c). Although the NMDA receptor agonists induce dopamine agonist-like behaviour as discussed in Chapter 1, the anxiogenic aspects of the effects of NMDA receptor antagonists do not seem to be well investigated.

7.4 Conclusion

The non-competitive NMDA receptor antagonist FR115427 impaired spatial learning in the water maze. The dose response and time course analysis suggested that this learning effect was not caused by a blocking effect on hippocampal LTP. The time course of the learning effect of FR115427 was similar to that of the induction of motor syndrome. As no direct effect on sensory motor system was observed in the poor learning performance following FR115427 treatment, the learning effect seemed to be related to the psychological effect of the drug underlying the induction of abnormal behaviour. A reduction of stress level during the water maze learning alleviate the learning impairment as well as the abnormal aversive behaviour induced by FR115427. Therefore, the anxiogenic-like effects of FR115427 is suggested to impair some psychologically important process of the learning in the water maze. More investigations need to be conducted on the psychological aspects of the effects of the NMDA receptor antagonists on learning.

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